

A Unified Framework for Preclusion of Multiple Steady States in Networks of Interacting Species

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Abstract

We present a unified framework for the preclusion of non-degenerate multiple steady states in a network of interacting species. Interaction networks are modeled via systems of ordinary differential equations in which the form of the species rate function is restricted by the reactions of the network and how the species influence each reaction. We characterize the set of interaction networks for which any choice of associated rate function is injective within each stoichiometric class and thus cannot exhibit multistationarity. Our criteria rely on the determinant of the Jacobian of the species rate functions that belong to the class of so-called general mass-action kinetics. The criteria are computationally tractable and easily implemented. Our approach embraces and extends much previous work on multistationarity, such as work in relation to chemical reaction networks with dynamics defined by mass-action or non-catalytic kinetics, and also work based on the graphical analysis of the interaction graph associated to the system.

Keywords: influence specification; reaction network; monotone kinetics; Jacobian; degenerate; general mass-action; interaction graph

1 Introduction

Interaction networks are used in many areas of science to represent the structural form of a dynamical system. This is in particular the case in systems biology and biochemistry where biochemical reactions are represented in the form of a network. However, similar network structures are also used in ecology, cell biology and epidemics, as well as outside the natural sciences, to describe the possible interactions or reactions between some species of interest. Common to these interaction networks is that they consist of a set of species and a set of

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interactions among the species. The state of the system is given by the concentration (or abundance) of each species and each interaction represents a transformation of the state of the system. An example is the chemical reaction $A + B \rightarrow 2C$ where one molecule of A and one molecule of B form two molecules of C .

Typically, a system of ordinary differential equations (ODEs) is used to describe how species concentrations change over time. The rate function of the system describes the instantaneous change in the concentrations when considering simultaneously the individual rates of all reactions in the network. Reaction rates are generally unknown but some qualitative aspects might be assumed and inferred. For instance, the presence of the species on the left side of a reaction (A, B in the above example) is a prerequisite for the reaction to take place and higher concentrations of these species typically lead to higher reaction rates. In some cases, reaction rates are fixed to follow a specific functional form that might depend on parameters to be inferred from experimental observations. In other cases, only weak assumptions are imposed on the reaction rates. We consider restrictions given by a so-called influence specification,²³ which specifies how each species affects (positively, negatively, or neutrally) the reaction rates. In this work an interaction network refers to the network (species and reactions) together with an influence specification.

For many networks the structure of the interactions and the influence specification alone determine dynamical and steady-state properties of the system (for example, multistationarity, persistence, or oscillations). That is to say, irrespectively the rate functions and the parameters quantifying them, taken together with the initial species concentrations, the system shows qualitatively the same type of behavior. It is perhaps surprising as the network structure itself does not encode any information about the specific rate functions and abundances. Even small networks might have many parameters which potentially could give rise to a rich and varied dynamics, as well as differences in the long-term behavior of the system.

Of particular interest has been to determine whether a system allows for multiple positive steady states, also known as multistationarity. Multistationarity provides a mechanism for switching (rapidly) between different responses and confers robustness to the steady-state values of the system.^{12, 18} For example, the growth (response) of a bacterial system depends on the availability of nutrients (stimulus). If the system exhibits multistationarity then fluctuations in the supply of nutrients determine the growth behavior. Within the range of nutrients where two (stable) steady states exist, the steady-state values are robust to minor fluctuations in the stimulus.

One way to address whether a system exhibits multistationarity is by finding the positive solutions to the steady-state equations of the system. Solving the equations might prove difficult, if not impossible, with difficulty depending on the assumptions about the reactions rates and the number of parameters. Here we take a more conceptual route and focus on understanding the characteristics of interaction networks that cannot exhibit multistationarity, irrespectively of the specific choices of reaction rates.

Various criteria have been developed to preclude the existence of multiple positive steady

states for general classes of reaction functions, also called kinetics, such as mass-action kinetics,^{4, 5, 7, 8} non-catalytic kinetics,² and weakly monotonic kinetics.²³ These criteria typically utilize the structure of the system together with some assumptions about the form of the rate functions. For example, mass-action kinetics assume that the reactions take place in a well-stirred container such that the frequency of collisions between species is proportional to their abundances. The reaction $2A + B \rightarrow D$ would have a reaction rate proportional to the product of the species concentrations, $c_A^2 c_B$, and the steady-state equations become a system of polynomial equations. Capitalizing on the polynomial form of the equations has lead to specific conditions to preclude multistationarity.^{4, 5, 8}

The aim of this paper is to provide a unified framework for *injectivity* of a network while allowing the kinetics to be of a general form but respecting an influence specification. Injectivity refers to injectivity of the (species formation) rate function that governs the dynamical behavior of the system. If this function is injective for the allowed kinetics then the system, or network, does not have the capacity for multiple positive steady states. The idea of injectivity was introduced in Ref. [4] but it is also underlying previous work on the preclusion of multistationarity.²⁴ We show that injectivity of an interaction network is closely related to injectivity of an interaction network taken with *general mass-action kinetics*, introduced in Ref. [11]. General mass-action kinetics are a generalization of mass-action kinetics and confer greater flexibility in the form of the rate functions than mass-action kinetics. Ref. [21] emphasizes the importance of general mass-action kinetics and the closely related fractal kinetics in biochemistry but their importance outside biology for modeling purposes is also well documented.^{1, 25} Both are based on a power-law formalism and it can be argued that they provide approximations to kinetics in general.²¹ The latter is also exemplified in our work.

General mass-action kinetics have many things in common with mass-action kinetics and parallel results can be derived for the two types of kinetics. We derive necessary and sufficient *determinant criteria* for a network to be injective over different classes of general mass-action kinetics, defined by influence specifications. The determinant refers to the determinant of a *modified* version of the species formation rate function (Definition 5.2 in this paper). We show that the determinant of the Jacobian of the modified function is non-zero for all concentration vectors and kinetics in one of the classes if and only if the network is injective over the particular class. The results utilize and extend parallel results for mass-action kinetics.⁸ The determinant criteria depend in part on the network structure and in part on the influence specification.

These criteria are of importance for several reasons. First of all, they provide means to preclude multiple positive steady states for classes of general mass-action kinetics. Secondly, they are easily computable using symbolic software packages such as Mathematica or Matlab and, thus, they are of practical use. Importantly, the set of interaction networks that are injective for all choices of general mass-action kinetics agrees with the set of interaction networks that are injective for all choices of kinetics that are in accordance with the influence specification. Therefore, our determinant criteria determine injectivity more broadly.

This work extends and embraces previous determinant criteria developed for networks taken

with mass-action kinetics.^{4, 5, 8} In Ref. [23], the authors also characterize networks that are injective for classes of kinetics defined by an influence specification (defined in a slightly different way than in this paper). The criteria discussed in this paper are different from the criteria in Ref. [23] and we extend their results. Further, our work clarifies the role played by general mass-action kinetics in deciding injectivity.

In Ref. [2] the authors study injectivity of a broad class of kinetics by putting some restrictions on the form of the reactions. Namely, reactions of the form $S \rightarrow 0$ belong to the network for all the species S and kinetics are the so-called non-catalytic kinetics. Interestingly, under their assumptions one of our determinant criteria for injectivity follows from their criterion. Further, in Ref. [2] injectivity of the network is derived by providing a criterion that guarantees that the Jacobian of the species formation rate function is a P -matrix (defined in Section 10). We extend this result to other classes of kinetics and arbitrary networks and show that the Jacobian of the modified species formation rate function is a P -matrix if and only if the function is injective.

Finally, this work also relates to a criterion for multistationarity based on the interaction graph.^{16, 24} The interaction graph records the sign of the entries in the Jacobian of a dynamical system and does not assume the existence of an underlying network structure as in our case. We show that their criterion agrees with one of our determinant criteria applied to a certain type of interaction networks, namely, those consisting exclusively of reactions of the form $S \rightarrow 0$ and $0 \rightarrow S$. We show that the existence of multiple non-degenerate steady states in the class of dynamical systems considered in Ref. [16, 24] is precluded by our injectivity criterion for these interaction networks. It follows that the results presented here are stronger since the information provided by the network structure enables to preclude multistationarity for a broader class of dynamical systems.

The structure of the paper is the following. In Section 2 we introduce some notation and in Section 3 we introduce the basic concepts of networks and kinetics. In Section 4, we discuss interaction networks and give examples from the literature. Section 5 introduces the notion of degeneracy and injectivity, two key concepts. Section 6 focusses on general mass-action kinetics. In Section 7, we derive a number of results on injectivity for different classes of general mass-actions kinetics. These results are used in Sections 8 and 9 and extended to broader and more general classes of kinetics. Section 9 includes also the relationship of our work to Ref. [23]. In Section 10, we develop the relationship to the work of Ref. [2] and in Section 11, we relate our work to that of Ref. [16]. Finally, in Section 12 we show that other types of kinetics could be used in place of general mass-action kinetics. To keep the exposition clear in the main text, all proofs are in the Appendix.

2 Notation

Let \mathbb{R}_+ denote the set of positive real numbers (without zero) and $\overline{\mathbb{R}}_+$ the set of non-negative real numbers (with zero). Similarly, let $\overline{\mathbb{N}}$ be the set of non-negative integers. Given a finite set \mathcal{E} , let $\overline{\mathbb{N}}^{\mathcal{E}}$ be the semi-ring of formal sums $v = \sum_{E \in \mathcal{E}} \lambda_E E$, with $\lambda_E \in \overline{\mathbb{N}}$. If $\lambda_E \in \mathbb{N}$ for all $E \in \mathcal{E}$, then we write $v \in \mathbb{N}^{\mathcal{E}}$. The semi-rings $\overline{\mathbb{R}}_+^{\mathcal{E}}$ and $\mathbb{R}_+^{\mathcal{E}}$ are defined analogously.

The ring of polynomials in \mathcal{E} is denoted $\mathbb{R}[\mathcal{E}]$. The total degree of a monomial $\prod_{E \in \mathcal{E}} E^{n_E}$, with n_E a non-negative integer for all E , is the sum of the degrees of the variables, $\sum_{E \in \mathcal{E}} n_E$. The degree of a polynomial is the maximum of the total degrees of its monomials.

If a polynomial p vanishes for all assignments $a: \mathcal{E} \rightarrow \mathbb{R}_+$ then $p = 0$ identically. Further, if p is a non-zero polynomial in $\mathbb{R}[\mathcal{E}]$ such that the degree of each variable in each monomial is either 1 or zero, then all the coefficients of p are non-negative if and only if $p(a(\mathcal{E})) > 0$ for any assignment $a: \mathcal{E} \rightarrow \mathbb{R}_+$.

For a vector $v = (v_1, \dots, v_m) \in \mathbb{R}^m$, let the positive part be $v_+ = (\max(v_1, 0), \dots, \max(v_m, 0))$ and the negative part be $v_- = (\min(v_1, 0), \dots, \min(v_m, 0))$. The support of v is defined as the set of indices for which v is non-zero, $\text{supp}(v) = \{i | v_i \neq 0\}$. The positive support of v is $\text{supp}^+(v) = \text{supp}(v_+)$ and the negative support is $\text{supp}^-(v) = \text{supp}(v_-)$. Let v^t denote the transpose of v and $u \cdot v$ the usual scalar product in \mathbb{R}^n .

For every $x \in \mathbb{R}$, we let $\text{sign}(x) \in \{-1, 0, 1\}$ be defined as

$$\text{sign}(x) = \begin{cases} -1 & \text{if } x < 0, \\ 0 & \text{if } x = 0, \\ 1 & \text{if } x > 0. \end{cases}$$

3 Networks as dynamical systems

In this section we introduce networks and kinetics, and associate a dynamical system with a network and a kinetics. The definition of a network is identical to that of a chemical reaction network, which is used mainly in (bio)chemistry to describe networks of (bio)chemical reactions.⁶ In general we use the nomenclature that is standard for chemical reaction networks. See for instance Ref. [6, 7] for background and extended discussions.

Definition 3.1. A *network* \mathcal{N} consists of three finite sets:

- (1) A set \mathcal{S} of *species*.
- (2) A set $\mathcal{C} \subset \overline{\mathbb{N}}^{\mathcal{S}}$ of *complexes*.
- (3) A set $\mathcal{R} \subset \mathcal{C} \times \mathcal{C}$ of *reactions*, such that $(y, y) \notin \mathcal{R}$ for all $y \in \mathcal{C}$, and if $y \in \mathcal{C}$, then there exists $y' \in \mathcal{C}$ such that either $(y, y') \in \mathcal{R}$ or $(y', y) \in \mathcal{R}$.

A network is denoted by $\mathcal{N} = (\mathcal{S}, \mathcal{C}, \mathcal{R})$.

We use the convention that an element $r = (y, y') \in \mathcal{R}$ is denoted by $r: y \rightarrow y'$. The *reactant* and the *product* (complexes) of a reaction $r: y \rightarrow y'$ are y and y' , respectively. By definition, any complex is either the reactant or the product of some reaction. The zero complex $0 \in \mathcal{C}$ is allowed by definition. A reaction $S \rightarrow 0$, $S \in \mathcal{S}$, is called an *outflow reaction*.

Throughout the paper, we use n to denote the number of species in \mathcal{S} . We fix an order in \mathcal{S} so that $\mathcal{S} = \{S_1, \dots, S_n\}$ and identify $\bar{\mathbb{N}}^{\mathcal{S}}$ with $\bar{\mathbb{N}}^n$. The species S_i is identified with the i -th canonical n -tuple of $\bar{\mathbb{N}}^n$ with 1 in the i -th position and zeroes elsewhere. Accordingly, a complex $y \in \mathcal{C}$ is given as $y = \sum_{i=1}^n y_i S_i$ or (y_1, \dots, y_n) . We assume that $y \in \bar{\mathbb{N}}^n$ as reactions typically involve integer numbers of species. However, the results presented in this paper hold generally for $y \in \mathbb{R}^n$. In examples we will often use other letters than S_i for species to ease the presentation.

Example 3.2. Enzyme biology provides a rich source of examples. For instance, consider the network with set of biochemical species $\mathcal{S} = \{S_1, S_2, S_3, S_4, S_5, S_6\}$, set of complexes $\{S_1 + S_3, S_1 + S_4, S_2 + S_4, S_2 + S_3, S_5, S_6\}$ and reactions



That is, the reactions are $r_1: S_1 + S_3 \rightarrow S_5$, $r_2: S_5 \rightarrow S_1 + S_3$, $r_3: S_5 \rightarrow S_1 + S_4$, $r_4: S_2 + S_4 \rightarrow S_6$, $r_5: S_6 \rightarrow S_2 + S_4$ and $r_6: S_6 \rightarrow S_2 + S_3$. This network is a main building block in protein modification systems and is known as the *futile cycle*. It assumes the Michaelis-Menten enzyme mechanism in which a substrate S_3 is modified into a substrate S_4 through the formation of an intermediate S_5 . The reaction is catalyzed by an enzyme S_1 . The modification can be reversed via a similar set of reactions with an intermediate S_6 and an enzyme S_2 .

Reactions in a network are schematic representations of dynamical processes. Over time the concentrations or abundances of the species in the network change as a consequence of the reactions. In order to describe the dynamical properties of the network we introduce a kinetics (Definition 3.3) and the species formation rate function (Definition 3.7). The kinetics provides the reaction rate for given species concentrations and the species formation rate function the instantaneous change in the concentrations when considering simultaneously the rate of all reactions.

Definition 3.3. A *kinetics* for a network $\mathcal{N} = (\mathcal{S}, \mathcal{C}, \mathcal{R})$ is an assignment to each reaction $y \rightarrow y' \in \mathcal{R}$ of a rate function $K_{y \rightarrow y'}: \Omega_K \rightarrow \mathbb{R}_+$, where Ω_K is a set such that $\mathbb{R}_+^n \subseteq \Omega_K \subseteq \mathbb{R}_+^n$ and

$$K_{y \rightarrow y'}(c) \geq 0 \quad \text{for all } c \in \Omega_K.$$

A kinetics for a network \mathcal{N} is denoted by $K = (K_{y \rightarrow y'})_{y \rightarrow y' \in \mathcal{R}}$ and the set of kinetics for \mathcal{N} by $\mathcal{K}(\mathcal{N})$. If $K_{y \rightarrow y'}$ is differentiable for all $y \rightarrow y' \in \mathcal{R}$ and $c \in \mathbb{R}_+^n$ then K is said to be a *differentiable kinetics*. The set of differentiable kinetics for \mathcal{N} is denoted by $\mathcal{K}_d(\mathcal{N})$.

Example 3.4. Let $\mathcal{N} = (\mathcal{S}, \mathcal{C}, \mathcal{R})$ be the network with $\mathcal{S} = \{S_1, S_2, S_3\}$, $\mathcal{C} = \{S_1 + S_2, S_3\}$ and \mathcal{R} given by the reaction $S_1 + S_2 \rightarrow S_3$. The kinetics $K \in \mathcal{K}(\mathcal{N})$ defined by $K_{S_1+S_2 \rightarrow S_3}(c) = kc_1/((\beta + c_1)c_2^\alpha)$, where k, α, β are positive constants has $\Omega_K = \overline{\mathbb{R}} \times \mathbb{R}_+ \times \overline{\mathbb{R}}_+$.

Example 3.5. Kinetics commonly used in chemistry and biology are the so-called mass-action kinetics. These were introduced by Guldberg and Waage in the 19th century based on the ideal assumption that the rate of a reaction is proportional to the product of the concentrations of the reactant species.³ Specifically, each reaction $y \rightarrow y'$ is assigned a positive constant $k_{y \rightarrow y'} \in \mathbb{R}_+$ and the rate function for the reaction is given by

$$K_{y \rightarrow y'}(c) = k_{y \rightarrow y'} \prod_{i=1}^n c_i^{y_i}$$

with $\Omega_K = \overline{\mathbb{R}}_+^n$ and $K = (K_{y \rightarrow y'})_{y \rightarrow y' \in \mathcal{R}}$. Under *in vivo* conditions, however, the use of mass-action kinetics might not be fully justified. Reactant species might not form a homogeneous mixture, for instance because they appear in too low concentrations or because their distribution depends on spatial constraints. In situations in which the use of mass-action is not justified, the use of other types of kinetics such as *general mass-action kinetics* or *Hill-type kinetics* are often preferred. These will be introduced later. Note that $K_{y \rightarrow y'}(c)$ is an increasing function in c_i and does not depend on other species than those involved in y .

Definition 3.6. The *stoichiometric subspace* of a network $\mathcal{N} = (\mathcal{S}, \mathcal{C}, \mathcal{R})$ is the following vector subspace of \mathbb{R}^n :

$$\Gamma = \langle y' - y \mid y \rightarrow y' \in \mathcal{R} \rangle.$$

Two vectors $c, c' \in \mathbb{R}^n$ are called *stoichiometrically compatible* if $c - c' \in \Gamma$, or equivalently, if $\omega \cdot c = \omega \cdot c'$ for all $\omega \in \Gamma^\perp$.

We let s be the dimension of Γ and, thus, the dimension of Γ^\perp is $d = n - s$. Note that Γ is independent of the choice of kinetics and depends only on the structure of the reactions. Being stoichiometrically compatible is an equivalence relation which partitions $\overline{\mathbb{R}}_+^n$ into classes, called *stoichiometric classes*. In particular, the stoichiometric class of a concentration vector $c \in \overline{\mathbb{R}}_+^n$ is $\{c + \Gamma\} \cap \overline{\mathbb{R}}_+^n$. Knowing the value of $\omega \cdot c$ for all vectors ω in a basis of Γ^\perp determines the stoichiometric class to which c belongs to. The value of $\omega \cdot c$ is called a *conserved amount*.

Definition 3.7. The *species formation rate function* for a network $\mathcal{N} = (\mathcal{S}, \mathcal{C}, \mathcal{R})$ with kinetics $K \in \mathcal{K}(\mathcal{N})$ and stoichiometric subspace Γ is the map $f_K: \Omega_K \rightarrow \Gamma$ defined by

$$f_K(c) = \sum_{y \rightarrow y' \in \mathcal{R}} K_{y \rightarrow y'}(c)(y' - y).$$

The dynamics of a network \mathcal{N} with kinetics $K \in \mathcal{K}(\mathcal{N})$ is described by a set of ordinary differential equations (ODEs) given by the species formation rate function:

$$\dot{c} = f_K(c),$$

where $\dot{c} = \dot{c}(t)$ denotes the derivative of $c(t)$ with respect to t . The *steady states* of the network are the solutions to the system of equations in c_1, \dots, c_n obtained by setting the derivatives of the concentrations to zero:

$$0 = f_K(c).$$

This system of equations is referred to as the steady-state equations. In particular, we are interested in the positive steady states, that is, the solutions c to the steady-state equations such that all concentrations are positive, $c \in \mathbb{R}_+^n$. Observe that the image of f_K is contained in Γ and it follows that for any $\omega \in \Gamma^\perp$ we have that $\omega \cdot \dot{c} = 0$. Therefore, $\omega \cdot c$ is independent of time and determined by the initial concentrations of the system.

For any differentiable function $f = (f_1, \dots, f_q): \Omega \rightarrow \mathbb{R}^q$ defined on a set Ω including \mathbb{R}_+^m , let $J_c(f)$ denote the Jacobian of f at $c \in \mathbb{R}_+^m$, that is, the $q \times m$ matrix with entry (i, j) being $\partial f_i(c)/\partial c_j$. If $K \in \mathcal{K}_d(\mathcal{N})$ is a differentiable kinetics then the Jacobian $J_c(f_K): \mathbb{R}_+^n \rightarrow \Gamma$ of f_K evaluated at $c \in \mathbb{R}_+^n$ is given for any $\gamma = (\gamma_1, \dots, \gamma_n) \in \mathbb{R}_+^n$ by

$$J_c(f_K)(\gamma) = \sum_{i=1}^n \sum_{y \rightarrow y' \in \mathcal{R}} \left(\frac{\partial K_{y \rightarrow y'}}{\partial c_i}(c) \cdot \gamma_i \right) (y' - y). \quad (3.8)$$

Example 3.9. The stoichiometric subspace of the futile cycle in Example 3.2 is

$$\Gamma = \langle S_1 + S_3 - S_5, S_5 - S_1 - S_4, S_2 + S_4 - S_6 \rangle$$

and has dimension $s = 3$. The dimension of Γ^\perp is $d = n - s = 3$ and a basis is $\{\omega^1, \omega^2, \omega^3\}$ with

$$\omega^1 = S_1 + S_5, \quad \omega^2 = S_2 + S_6, \quad \omega^3 = S_3 + S_4 + S_5 + S_6. \quad (3.10)$$

If $K \in \mathcal{K}(\mathcal{N})$ is any kinetics then the corresponding system of ODEs for the futile cycle is:

$$\begin{aligned} \dot{c} = & K_{S_1+S_3 \rightarrow S_5}(c)(-1, 0, -1, 0, 1, 0) + K_{S_5 \rightarrow S_1+S_3}(c)(1, 0, 1, 0, -1, 0) \\ & + K_{S_5 \rightarrow S_1+S_4}(c)(1, 0, 0, 1, -1, 0) + K_{S_2+S_4 \rightarrow S_6}(c)(0, -1, 0, -1, 0, 1) \\ & + K_{S_6 \rightarrow S_2+S_4}(c)(0, 1, 0, 1, 0, -1) + K_{S_6 \rightarrow S_2+S_3}(c)(0, 1, 1, 0, 0, -1), \end{aligned}$$

which written component-wise yields the system

$$\begin{aligned}
\dot{c}_1 &= -K_{S_1+S_3 \rightarrow S_5}(c) + K_{S_5 \rightarrow S_1+S_3}(c) + K_{S_5 \rightarrow S_1+S_4}(c) \\
\dot{c}_2 &= -K_{S_2+S_4 \rightarrow S_6}(c) + K_{S_6 \rightarrow S_2+S_4}(c) + K_{S_6 \rightarrow S_2+S_3}(c) \\
\dot{c}_3 &= -K_{S_1+S_3 \rightarrow S_5}(c) + K_{S_5 \rightarrow S_1+S_3}(c) + K_{S_6 \rightarrow S_2+S_3}(c) \\
\dot{c}_4 &= K_{S_5 \rightarrow S_1+S_4}(c) - K_{S_2+S_4 \rightarrow S_6}(c) + K_{S_6 \rightarrow S_2+S_4}(c) \\
\dot{c}_5 &= K_{S_1+S_3 \rightarrow S_5}(c) - K_{S_5 \rightarrow S_1+S_3}(c) - K_{S_5 \rightarrow S_1+S_4}(c) \\
\dot{c}_6 &= K_{S_2+S_4 \rightarrow S_6}(c) - K_{S_6 \rightarrow S_2+S_4}(c) - K_{S_6 \rightarrow S_2+S_3}(c).
\end{aligned}$$

Observe that $\dot{c}_1 + \dot{c}_5 = \dot{c}_2 + \dot{c}_6 = \dot{c}_3 + \dot{c}_4 = \dot{c}_5 + \dot{c}_6 = 0$ for any kinetics $K \in \mathcal{K}(\mathcal{N})$.

4 Interaction networks and influence specifications

Here we introduce interaction networks and some concepts related to them. An interaction network consists of two parts: a network and an influence specification. The latter stipulates structurally what species influence the rate of a reaction and whether a species acts as an inducer or an inhibitor of a reaction. We use influence specifications to restrict the set of possible kinetics for a network. Our definition of an influence specification is inspired by Ref. [23].

Definition 4.1. An *influence specification* for a network $\mathcal{N} = (\mathcal{S}, \mathcal{C}, \mathcal{R})$ is an assignment to each reaction $y \rightarrow y' \in \mathcal{R}$ of a function $I_{y \rightarrow y'} : \mathcal{S} \rightarrow \{-1, 0, 1\}$. An influence specification for \mathcal{N} is denoted by $\mathcal{I} = (I_{y \rightarrow y'})_{y \rightarrow y' \in \mathcal{R}}$. We define the following distinguished influence specifications:

- The *complex dependent influence specification*, denoted by $\mathcal{I}_{\mathcal{C}}$, is defined as the influence specification with $I_{y \rightarrow y'}(S_i) = 1$ for $i \in \text{supp}(y)$ and $I_{y \rightarrow y'}(S_i) = 0$ for $i \notin \text{supp}(y)$, for all $y \rightarrow y' \in \mathcal{R}$.
- The *reaction dependent influence specification*, denoted by $\mathcal{I}_{\mathcal{R}}$, is defined as the influence specification with $I_{y \rightarrow y'}(S_i) = 1$ for $i \in \text{supp}(y)$, $I_{y \rightarrow y'}(S_i) = -1$ for $i \in \text{supp}(y') \setminus \text{supp}(y)$ and $I_{y \rightarrow y'}(S_i) = 0$ for $i \notin \text{supp}(y') \cup \text{supp}(y)$, for all $y \rightarrow y' \in \mathcal{R}$.

The species S_i with $I_{y \rightarrow y'}(S_i) \neq 0$ influence the reaction $y \rightarrow y'$. Those with $I_{y \rightarrow y'}(S_i) = 1$ are assumed to have positive influence or enhance the reaction while those with $I_{y \rightarrow y'}(S_i) = -1$ are assumed to have negative influence and an inhibitory effect on the reaction. Those with $I_{y \rightarrow y'}(S_i) = 0$ have neutral influence.

Remark 4.2. In typical applications an influence specification satisfies

$$I_{y \rightarrow y'}(S_i) \neq 0, \quad \text{for all } i \in \text{supp}(y).$$

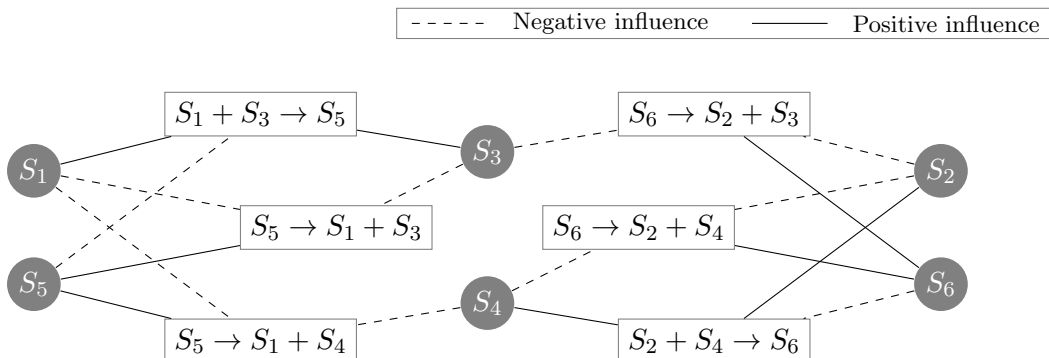


Figure 1: Reaction dependent influence specification for the futile cycle, drawn as a bipartite graph. There is an edge between a species and a reaction if the species has non-zero influence on the reaction. If the influence is positive, the edge is solid. If the influence is negative, we draw a dashed edge. The complex dependent influence specification is in the case obtained by removing the dashed edges. The species interacting in a reaction, for example $S_5 \rightarrow S_1 + S_4$ cannot be read off from the edges.

That is, the enhancers and inhibitors include all species involved in the reactant complex y . We do not impose this restriction here because the condition is not required for the mathematical development of the work. In chemical reaction theory it is typically required that the species in the reactant complex have positive influence on the reaction^{10, 23} and not negative or neutral, as in Definition 4.1. However, relaxation of this assumption is found^{11, 21} and if some species in some reactions are only partially known, as might be the case in some biochemical systems, this appears reasonable.

Definition 4.3. An *interaction network* $(\mathcal{N}, \mathcal{I})$ is a network $\mathcal{N} = (\mathcal{S}, \mathcal{C}, \mathcal{R})$ together with an influence specification \mathcal{I} .

If no species appear both in the reactant and the product of a reaction then the reaction dependent influence specification $\mathcal{I}_{\mathcal{R}}$ is given by the functions $\sigma_{y \rightarrow y'}(S_i) = \text{sign}(y_i - y'_i)$, $y \rightarrow y' \in \mathcal{R}$. However, if this is not the case then $\sigma_{y \rightarrow y'}$, $y \rightarrow y' \in \mathcal{R}$, might not define the reaction dependent influence specification. For instance, the reaction $S_1 + S_2 \rightarrow S_1 + S_3$ gives $\sigma_{y \rightarrow y'}(S_1) = 0$, contradicting that $\mathcal{I}_{\mathcal{R}}(S_1) = 1$.

It is sometimes useful to illustrate an influence specification with a labeled bipartite graph with node set $\mathcal{S} \cup \mathcal{R}$. We draw a positive edge between a species and a reaction if the species has positive influence over the reaction. We draw a negative edge if the species has negative influence on the reaction. An example using the futile cycle and the reaction dependent influence specification is illustrated in Figure 1.

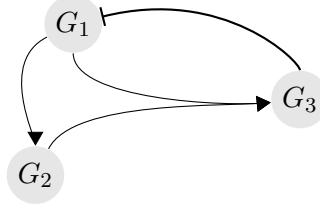


Figure 2: The influence specification for the transcription of the three genes are shown. The presence of G_3 reduces the production of G_1 , whereas the presence of G_1 and G_2 cooperatively induce the production of G_3 . Likewise G_1 induces the production of G_2 .

Example 4.4. Common examples in the literature are gene regulatory networks.¹⁵ These are typically represented by diagrams as the one shown in Figure 2. The diagram represents three genes, each transcribing a protein. The proteins mutually affect the transcription rates of the genes such that the genes influence each other. The diagram corresponds to the interaction network with production reactions

$$0 \longrightarrow G_i \quad i = 1, 2, 3,$$

and influence specification $\mathcal{I} = (I_{y \rightarrow y'})_{y \rightarrow y' \in \mathcal{R}}$ with (only non-zero values are listed)

$$I_{0 \rightarrow G_1}(G_3) = -1, \quad \text{and} \quad I_{0 \rightarrow G_2}(G_1) = I_{0 \rightarrow G_3}(G_1) = I_{0 \rightarrow G_3}(G_2) = 1.$$

In the following, we assume that \mathcal{S} , \mathcal{C} , and \mathcal{R} denote the sets of species, complexes and reactions, respectively, of a network \mathcal{N} , unless otherwise specified. Let $(\mathcal{N}, \mathcal{I})$ be an interaction network. For $y \rightarrow y' \in \mathcal{R}$, define

$$I_{y \rightarrow y'}^+ = \{i \mid I_{y \rightarrow y'}(S_i) = 1\}, \quad I_{y \rightarrow y'}^- = \{i \mid I_{y \rightarrow y'}(S_i) = -1\}, \quad \text{and} \quad I_{y \rightarrow y'}^0 = \{i \mid I_{y \rightarrow y'}(S_i) = 0\}.$$

Two concentration vectors $a, b \in \overline{\mathbb{R}}_+^n$ are said to be *non-overlapping with respect to \mathcal{I}* ,

$$\text{if } I_{y \rightarrow y'}^+ \not\subseteq \text{supp}(a) \quad \text{implies} \quad I_{y \rightarrow y'}^+ \subseteq \text{supp}(b)$$

for all reactions $y \rightarrow y'$. That is, the coordinates a_i, b_j (potentially with $i = j$) cannot both be zero if i, j are both in $I_{y \rightarrow y'}^+$ for some $y \rightarrow y'$. When it is clear from the context what influence specification we are referring to, we omit “with respect to \mathcal{I} ” and just say that a, b are non-overlapping. Non-overlapping is a concept that specifies how two concentration vectors on the boundary of the positive orthant are positioned with respect to each other in relation to an influence specification \mathcal{I} . In particular, if one or both of the concentration vectors a, b are positive then they are non-overlapping.

Definition 4.5. A kinetics $K = (K_{y \rightarrow y'})_{y \rightarrow y' \in \mathcal{R}}$ for a network $\mathcal{N} = (\mathcal{S}, \mathcal{C}, \mathcal{R})$ respects an influence specification \mathcal{I} if, for all $c \in \Omega_K$,

$$K_{y \rightarrow y'}(c) > 0 \quad \text{if and only if} \quad I_{y \rightarrow y'}^+ \subseteq \text{supp}(c).$$

If a kinetics respects an influence specification then a reaction occurs if and only if all species with positive influence on the reaction are present (that is, they are in positive concentrations). If one species is not present then the reaction cannot occur. Absence of species with negative or neutral influence does not prevent the reaction from taking place.

Let $\Omega_K(I_{y \rightarrow y'}^+) := \{c \in \Omega_K \mid I_{y \rightarrow y'}^+ \subseteq \text{supp}(c)\} = \{c \in \Omega_K \mid K_{y \rightarrow y'}(c) \neq 0\}$ denote the set of concentration vectors for which the kinetics $K_{y \rightarrow y'}$ does not vanish.

Definition 4.6. Let $K = (K_{y \rightarrow y'})_{y \rightarrow y' \in \mathcal{R}}$ be a kinetics for a network $\mathcal{N} = (\mathcal{S}, \mathcal{C}, \mathcal{R})$ that respects an influence specification \mathcal{I} . We say that K is *strictly monotonic with respect to \mathcal{I}* if for all reactions $y \rightarrow y' \in \mathcal{R}$ and $i = 1, \dots, n$, the restriction of the function $K_{y \rightarrow y'}(\cdot)$ to $\Omega_K(I_{y \rightarrow y'}^+)$ is

- (i) strictly increasing in c_i if $i \in I_{y \rightarrow y'}^+$.
- (ii) strictly decreasing in c_i if $i \in I_{y \rightarrow y'}^-$.
- (iii) constant in c_i if $i \in I_{y \rightarrow y'}^0$.

Let $\mathcal{K}(\mathcal{N}, \mathcal{I})$ denote the set of kinetics that are strictly monotonic with respect to the influence specification \mathcal{I} .

The definition says that the rate functions $K_{y \rightarrow y'}$ are strictly monotonic or constant in the coordinate c_i whenever the remaining coordinates take positive values for the species with positive influence. Strictly monotonic refers to the kinetics, but this does not imply that the species formation rate function is monotone.

Example 4.7. The length of a larvae is often assumed to increase linearly with a slow down in the growth rate as the length increases. Denoting by c the length of the larvae, one model of the length is $\dot{c} = \alpha_1(\alpha_2 + c)$, where α_1, α_2 are positive constants. This system arises from the interaction network with reaction $S \rightarrow 2S$ (the addition of new cells at the two ends of the larvae) and kinetics $K_{S \rightarrow 2S}(c) = \alpha_1/(\alpha_2 + c)$. This kinetics is strictly monotonic with respect to the influence specification given by $I_{S \rightarrow 2S}(S) = -1$ (compare Remark 4.2).

Lemma 4.8. Let $K = (K_{y \rightarrow y'})_{y \rightarrow y' \in \mathcal{R}}$ be a kinetics for a network \mathcal{N} that respects an influence specification \mathcal{I} . Then, K is strictly monotonic with respect to \mathcal{I} if and only if for each pair of non-overlapping concentration vectors $a, b \in \Omega_K$, the following implications hold for all reactions $y \rightarrow y'$:

- (i) if $K_{y \rightarrow y'}(a) > K_{y \rightarrow y'}(b)$ then $S_i \in \mathcal{S}$ with $\text{sign}(a_i - b_i) = I_{y \rightarrow y'}(S_i) \neq 0$ for some i .
- (ii) if $K_{y \rightarrow y'}(a) = K_{y \rightarrow y'}(b)$ then either $a_i = b_i$ for all $i \in I_{y \rightarrow y'}^+ \cup I_{y \rightarrow y'}^-$, or $\text{sign}(a_i - b_i) = I_{y \rightarrow y'}(S_i) \neq 0$ and $\text{sign}(a_j - b_j) = -I_{y \rightarrow y'}(S_j) \neq 0$ for some distinct i, j .

Example 4.9. A standard model of population growth is¹⁹

$$\dot{c} = rc \left(1 - \frac{c}{K}\right), \quad (4.10)$$

where c denotes the size of a population S and r, K are positive constants. The system can be considered an interaction network \mathcal{N} with two opposing reactions $S \rightarrow 2S$ (birth) and $S \rightarrow 0$ (death), $\mathcal{S} = \{S\}$, $\mathcal{C} = \{S, 2S, 0\}$, and influence specification $I_{S \rightarrow 2S}(S) = I_{S \rightarrow 0}(S) = 1$. The kinetics with $K_{S \rightarrow 2S}(c) = rc$ and $K_{S \rightarrow 0}(c) = rc^2/K$ is strictly monotonic with respect to \mathcal{I} and gives equation (4.10). The system has a stable steady state at $c = K$ and an unstable steady state at $c = 0$. The two steady states are non-overlapping with respect to \mathcal{I} .

This population model has been generalized in various ways, also incorporating competition among different populations.¹⁹ Any model of one population where \dot{c} initially increases in c until a certain point and then decreases can be seen as giving a strictly monotonic kinetics for \mathcal{N} . One particular instance of a competing species model is shown in Example 4.13.

Definition 4.11. A kinetics $K = (K_{y \rightarrow y'})_{y \rightarrow y' \in \mathcal{R}}$ for a network \mathcal{N} is *differentiable with respect to an influence specification \mathcal{I}* if K respects \mathcal{I} and, for every reaction $y \rightarrow y' \in \mathcal{R}$, $K_{y \rightarrow y'}(\cdot)$ is continuous at $c \in \Omega_K$, differentiable at $c \in \mathbb{R}_+^n$, and for each i the partial derivative $\frac{\partial K_{y \rightarrow y'}}{\partial c_i}(c)$ has constant sign

$$I_{y \rightarrow y'}(S_i) = \text{sign} \left(\frac{\partial K_{y \rightarrow y'}}{\partial c_i}(c) \right)$$

in \mathbb{R}_+^n . Let $\mathcal{K}_d(\mathcal{N}, \mathcal{I})$ denote the set of kinetics that are differentiable with respect to the influence specification \mathcal{I} .

Note that $\mathcal{K}_d(\mathcal{N}, \mathcal{I})$ is *not* the intersection of $\mathcal{K}(\mathcal{N}, \mathcal{I})$ with $\mathcal{K}_d(\mathcal{N})$ as we require the sign of the partial derivatives to be constant in Definition 4.11, which is not implied by being strictly monotonic and differentiable. Any mass-action kinetics K belongs to $\mathcal{K}_d(\mathcal{N}, \mathcal{I}_C)$. We state without proof:

Lemma 4.12. *If $K \in \mathcal{K}_d(\mathcal{N}, \mathcal{I})$ then $K \in \mathcal{K}(\mathcal{N}, \mathcal{I})$.*

Example 4.13. An example from ecology is given by the Lotka-Volterra equations for modeling predator-prey dynamics or competing populations.^{19, 20} The modeling equations are

$$\dot{c}_1 = c_1(\alpha - \beta c_2), \quad \text{and} \quad \dot{c}_2 = -c_2(\gamma - \beta c_1),$$

where c_1 and c_2 are the abundance of two species S_1 (prey) and S_2 (predator). The system can be considered a network $\mathcal{N} = (\mathcal{S}, \mathcal{C}, \mathcal{R})$ with $\mathcal{S} = \{S_1, S_2\}$, $\mathcal{C} = \{0, S_1, S_2, 2S_1, 2S_2, S_1 + S_2\}$ and $\mathcal{R} = \{S_1 \rightarrow 2S_1, S_1 + S_2 \rightarrow 2S_2, S_2 \rightarrow 0\}$. Define a kinetics $K \in \mathcal{K}(\mathcal{N})$ by

$$K_{S_1 \rightarrow 2S_1}(c_1, c_2) = \alpha c_1, \quad K_{S_1 + S_2 \rightarrow 2S_2}(c_1, c_2) = \beta c_1 c_2, \quad \text{and} \quad K_{S_2 \rightarrow 0}(c_1, c_2) = \gamma c_2,$$

with $\alpha, \beta, \gamma > 0$. Then, with the complex dependent influence specification $\mathcal{I}_{\mathcal{C}}$, $(\mathcal{N}, \mathcal{I}_{\mathcal{C}})$ is an interaction network and $K \in \mathcal{K}(\mathcal{N}, \mathcal{I}_{\mathcal{C}})$ is a mass-action kinetics (Example 3.5). The species formation rate function is given by $f_K(c_1, c_2) = (c_1(\alpha - \beta c_2), -c_2(\gamma - \beta c_1))$. The stoichiometric subspace

$$\Gamma = \langle S_1, S_2 - S_1, -S_2 \rangle$$

has dimension 2. Hence $\Gamma^\perp = \{0\}$ and there are no conserved amounts. The system has one stable steady state $(c_1, c_2) = (\gamma/\beta, \alpha/\beta)$ and one unstable $(0, 0)$. It shows periodic oscillations.²⁰

The reactions with the given kinetics can be interpreted in the following way (here assuming prey are mice and predators are owls): the mice reproduce, the owls reproduce while feeding on mice (one mouse is consumed for each newborn owl) and owls die. Mice only die when consumed by owls. The mouse population finds ample food at all times and reproduce at a rate proportional to their abundance, whereas the reproduction rate of the owls depends linearly on the availability of mice. Lotka and Volterra proposed the model independently of each other as a model of species interaction.²⁰ In epidemics, the same model has been proposed as a model of disease dynamics with prey being individuals susceptible to disease and predators being infected individuals.^{1, 17} Also the model has appeared in physical chemistry as a model of H_2O_2 combustion.²²

Remark 4.14. The number and form of the reactions, as well as the influence specification, is not uniquely determined from the equations describing the system. In Example 4.13, the reaction $S_1 \rightarrow 2S_1$ could be replaced by $0 \rightarrow S_1$, but the interpretation of the reaction might change. Similarly, the second reaction $S_1 + S_2 \rightarrow 2S_2$ could be replaced by $S_2 \rightarrow 2S_2$, assuming that species S_1 has positive influence on the reaction.

Example 4.15. Ref. [13] used a model similar to that of Example 4.13 to study bacterial uptake of nutrients in microbial ecology. The modeling equations are

$$\dot{c}_1 = \frac{V c_1 c_2}{K + c_2}, \quad \text{and} \quad \dot{c}_2 = -\frac{V c_1 c_2}{Y(K + c_2)},$$

where Y, K, V are positive parameters and c_1, c_2 the concentrations of two species S_1, S_2 where S_1 is a bacteria that feeds on a (chemical) nutrient S_2 . The system can be represented as a network $\mathcal{N} = (\mathcal{S}, \mathcal{C}, \mathcal{R})$ with $\mathcal{S} = \{S_1, S_2\}$, $\mathcal{C} = \{0, S_1, S_2\}$ and \mathcal{R} given by



representing the clonal reproduction of the bacteria and the consumption of the nutrient. The kinetics K with $K_{S_1 \rightarrow 2S_1}(c_1, c_2) = \frac{V c_1 c_2}{K + c_2}$ and $K_{S_2 \rightarrow 0}(c_1, c_2) = \frac{V c_1 c_2}{Y(K + c_2)}$ is strictly monotonic with

respect to the influence specification \mathcal{I} with $I_{S_1 \rightarrow 2S_1}(S_1) = I_{S_1 \rightarrow 2S_1}(S_2) = 1$ and $I_{S_2 \rightarrow 0}(S_1) = I_{S_2 \rightarrow 0}(S_2) = 1$. All steady states have $c_1 = 0$ or $c_2 = 0$. Hence two steady states are non-overlapping with respect to \mathcal{I} if at least one of them is strictly positive. Further, all steady states are degenerate.

5 Degenerate steady states and injectivity

In this section some key concepts and definitions are introduced, namely that of degeneracy of a steady state and injectivity of a network.

Definition 5.1. Let $\mathcal{N} = (\mathcal{S}, \mathcal{C}, \mathcal{R})$ be a network and $K \in \mathcal{K}_d(\mathcal{N})$ a differentiable kinetics. A steady state $c \in \mathbb{R}_+^n$ of \mathcal{N} is *degenerate* if $\ker(J_c(f_K)) \cap \Gamma \neq \{0\}$.

Alternatively, a steady state is degenerate if the Jacobian restricted to the stoichiometric subspace Γ is non-singular. Denote the components of the species formation rate function by $f_K = (f_{K,1}, \dots, f_{K,n})$.

Definition 5.2. Let $\mathcal{N} = (\mathcal{S}, \mathcal{C}, \mathcal{R})$ be a network. A basis $\{\omega^1, \dots, \omega^d\}$ of Γ^\perp with $\omega^i = (\lambda_1^i, \dots, \lambda_n^i)$ is said to be *reduced* if $\lambda_i^i = 1$ for all i and $\lambda_j^i = 0$ for all $j = 1, \dots, \widehat{i}, \dots, d$. If $K \in \mathcal{K}(\mathcal{N})$ is a kinetics for \mathcal{N} then the *associated extended rate function* $\tilde{f}_K: \Omega_K \rightarrow \mathbb{R}^n$ is the function defined by

$$\tilde{f}_K(c) = (\omega^1 \cdot c, \dots, \omega^d \cdot c, f_{K,d+1}(c), \dots, f_{K,n}(c)).$$

After reordering of the species set if necessary, a reduced basis always exists for any network \mathcal{N} and is unique. Therefore, from now on, we fix that the set of species is ordered such that a reduced basis exists. It does not depend on the kinetics $K \in \mathcal{K}(\mathcal{N})$.

Example 5.3. The basis of Γ^\perp provided for the futile cycle in equation (3.10) is reduced. The associated extended rate function $\tilde{f}_K: \Omega_K \rightarrow \mathbb{R}^6$ for any kinetics $K \in \mathcal{K}(\mathcal{N})$ is

$$\begin{aligned} \tilde{f}_K(c) = & (c_1 + c_5, c_2 + c_6, c_3 + c_4 + c_5 + c_6, K_{S_5 \rightarrow S_1 + S_4}(c) - K_{S_2 + S_4 \rightarrow S_6}(c) + K_{S_6 \rightarrow S_2 + S_4}(c), \\ & K_{S_1 + S_3 \rightarrow S_5}(c) - K_{S_5 \rightarrow S_1 + S_3}(c) - K_{S_5 \rightarrow S_1 + S_4}(c), K_{S_2 + S_4 \rightarrow S_6}(c) - K_{S_6 \rightarrow S_2 + S_4}(c) - K_{S_6 \rightarrow S_2 + S_3}(c)). \end{aligned}$$

The next proposition is proved in Ref. [8] for mass-action kinetics. However, the proof applies generally to differentiable kinetics without changes.

Proposition 5.4. Let $\mathcal{N} = (\mathcal{S}, \mathcal{C}, \mathcal{R})$ be a network, $K \in \mathcal{K}_d(\mathcal{N})$ a differentiable kinetics, $\{\omega^1, \dots, \omega^d\}$ a reduced basis of Γ^\perp and $\tilde{f}_K(c)$ the associated extended rate function. If $c \in \mathbb{R}_+^n$ then

$$\ker(J_c(f_K)) \cap \Gamma = \{0\} \quad \text{if and only if} \quad \det(J_c(\tilde{f}_K)) \neq 0.$$

In particular, a steady state $c \in \mathbb{R}_+^n$ is degenerate if and only if $\det(J_c(\tilde{f}_K)) = 0$.

Finally, we introduce the notion of injectivity.

Definition 5.5. Let $\mathcal{N} = (\mathcal{S}, \mathcal{C}, \mathcal{R})$ be a network.

- (i) We say that \mathcal{N} is *injective* over $\mathcal{K}_0 \subseteq \mathcal{K}(\mathcal{N})$ if for any pair of distinct stoichiometrically compatible vectors $a, b \in \mathbb{R}_+^n$ we have $f_K(a) \neq f_K(b)$ for all $K \in \mathcal{K}_0$.
- (ii) If \mathcal{I} is an influence specification for \mathcal{N} , we say that \mathcal{N} is *\mathcal{I} -injective* over $\mathcal{K}_0 \subseteq \mathcal{K}(\mathcal{N}, \mathcal{I})$ if for any $K \in \mathcal{K}_0$ and any pair of distinct non-overlapping stoichiometrically compatible vectors $a, b \in \Omega_K$ we have $f_K(a) \neq f_K(b)$.

A network is said to have the *capacity for multiple positive steady states* over \mathcal{K}_0 if there exists a kinetics $K \in \mathcal{K}_0$ and distinct stoichiometrically compatible vectors $a, b \in \mathbb{R}_+^n$ such that $f_K(a) = f_K(b) = 0$. Clearly, if \mathcal{N} is injective over some appropriate set of kinetics \mathcal{K}_0 , then \mathcal{N} does not have the capacity for multiple steady states over \mathcal{K}_0 . If an influence \mathcal{I} for \mathcal{N} is specified and \mathcal{N} is \mathcal{I} -injective, then the existence of pairs of distinct non-overlapping stoichiometrically compatible steady states $a, b \in \Omega_K$ is also precluded. Therefore, the concept of non-overlapping vectors accounts for the occurrence of some types of multiple steady states on the boundary of Ω_K and not only multiple steady states in the interior of Ω_K .

Note that in Definition 5.5(ii) above, the concentration vectors a, b are required to be non-overlapping with respect to \mathcal{I} . This set of non-overlapping pairs might vary with K since Ω_K depends on $K \in \mathcal{K}_0$. Since pairs of positive concentration vectors are non-overlapping for any influence specification \mathcal{I} , \mathcal{I} -injectivity implies injectivity. In Ref. [23], the authors preclude the occurrence of pairs of distinct stoichiometrically compatible steady states a, b such that at least one of them is in the interior of Ω_K (which is taken to be \mathbb{R}_+^n). As noticed above, such pairs are non-overlapping and hence covered by our approach. In Ref. [8], the condition of non-overlapping is applied to networks with mass-action kinetics and influence specification $\mathcal{I}_{\mathcal{C}}$. It is possible to have $f_K(\tilde{a}) = f_K(\tilde{b})$ for a pair of *not* non-overlapping vectors and at the same time $f_K(a) \neq f_K(b)$ for all non-overlapping pairs.⁸

The rest of the paper is devoted to characterize the networks that are \mathcal{I} -injective for different families of kinetics and that consequently cannot have the capacity for multiple positive steady states. The aim is to provide a criterion for a network \mathcal{N} to be \mathcal{I} -injective over a set of kinetics $\mathcal{K}_0 \subseteq \mathcal{K}(\mathcal{N}, \mathcal{I})$ in terms of computational tractable quantities that rely exclusively on the network structure and the influence specification. To this end we introduce the class of general mass-action kinetics (defined in Section 6) and derive some injectivity results for classes of general mass-action kinetics using techniques introduced in Ref. [8]. Furthermore, in Section 8, we will relate injectivity of a network with general mass-action kinetics to \mathcal{I} -injectivity for the same network with kinetics that are strictly monotonic with respect to \mathcal{I} .

6 General mass-action kinetics

General mass-action kinetics form a large family of kinetics.¹¹ They are generalizations of mass-action kinetics and are based on a power-law formalism. Their general form make them flexible for modeling purposes in many areas of science such as chemistry, ecology and epidemics. As mentioned in Example 3.5 general mass-action kinetics might be used when mass-action kinetics are not suitable and, furthermore, they can be seen as an approximation to a wide range of other kinetics.²¹ Furthermore, these kinetics are important in the study of injectivity in that they, in some sense, are “dense” in the set of kinetics that are strictly monotonic with respect to an influence specification (to be made precise in Section 8). That is to say, injectivity of a network \mathcal{N} over certain sets of kinetics can be determined on the basis of injectivity of \mathcal{N} over suitable sets of general mass-action kinetics.

In this section we introduce networks with general mass-action kinetics. Networks with general mass-action kinetics have properties that are very similar to networks with mass-action kinetics and similar results regarding injectivity can be derived for general mass-action kinetics as for mass-action kinetics themselves.

For a concentration vector c and $v \in \mathbb{R}^n$, we associate the power-law $c^v = \prod_{i=1}^n c_i^{v_i}$. We define $0^0 = 1$ and $0^x = 0$ for any real number $x \neq 0$. For example, if $v = (2.1, 0.72, 0, -1) \in \mathbb{R}^4$, then the associated power-law is $c^v = c_1^{2.1} c_2^{0.72} c_4^{-1}$. A power-law is well defined for all $c \in \mathbb{R}_+^n$ such that $c_i > 0$ if $v_i < 0$.

Definition 6.1. Let $\mathcal{N} = (\mathcal{S}, \mathcal{C}, \mathcal{R})$ be a network. We define:

- (i) A *kinetic order* is a \mathcal{R} -tuple of vectors $\mathbf{v} = (v_{y \rightarrow y'})_{y \rightarrow y' \in \mathcal{R}}$ with $v_{y \rightarrow y'} \in \mathbb{R}^n$.
- (ii) A *rate vector* is a \mathcal{R} -tuple of scalars $\kappa = (k_{y \rightarrow y'})_{y \rightarrow y' \in \mathcal{R}}$ with $k_{y \rightarrow y'} \in \mathbb{R}_+$. The constant $k_{y \rightarrow y'}$ is called the *rate constant* of reaction $y \rightarrow y'$.

A kinetics $K = (K_{y \rightarrow y'})_{y \rightarrow y' \in \mathcal{R}} \in \mathcal{K}(\mathcal{N})$ is a *general mass-action kinetics* with rate constant κ and kinetic order \mathbf{v} if

$$K_{y \rightarrow y'}(c) = k_{y \rightarrow y'} c^{v_{y \rightarrow y'}}, \quad \text{for all } y \rightarrow y' \in \mathcal{R},$$

and Ω_K is the maximal common domain of definition of the power-laws $c^{v_{y \rightarrow y'}}$, $y \rightarrow y' \in \mathcal{R}$ in the positive orthant. For simplicity, we identify the pair (κ, \mathbf{v}) with the kinetics K . We let $\mathcal{K}_g(\mathcal{N})[\mathbf{v}]$ denote the set of general mass-action kinetics (κ, \mathbf{v}) with arbitrary κ but fixed \mathbf{v} and let $\mathcal{K}_g(\mathcal{N})$ denote the set of all general mass-action kinetics for the network \mathcal{N} .

Note that $k_{y \rightarrow y'}$ is a scalar while $v_{y \rightarrow y'}$ is a vector. By definition, general mass-action kinetics are differentiable kinetics, that is $\mathcal{K}_g(\mathcal{N}) \subseteq \mathcal{K}_d(\mathcal{N})$. Mass-action kinetics are special types of general mass-action kinetics obtained by considering the kinetic order with $v_{y \rightarrow y'} = y$ for all $y \rightarrow y' \in \mathcal{R}$. Therefore, if we let $\mathbf{y} = (y)_{y \rightarrow y' \in \mathcal{R}}$ then the set of mass-action kinetics for a network \mathcal{N} is $\mathcal{K}_g(\mathcal{N})[\mathbf{y}]$.

If $K = (\kappa, \mathbf{v}) \in \mathcal{K}_g(\mathcal{N})$ is a general mass-action kinetics for a network \mathcal{N} then the species formation rate function is denoted by $f_K = f_{\kappa, \mathbf{v}}$ and, for $c \in \Omega_K$, we have

$$f_{\kappa, \mathbf{v}}(c) = \sum_{y \rightarrow y' \in \mathcal{R}} k_{y \rightarrow y'} c^{v_{y \rightarrow y'}} (y' - y).$$

The Jacobian of $f_{\kappa, \mathbf{v}}$ with respect to $c \in \mathbb{R}_+^n$ takes the form

$$J_c(f_{\kappa, \mathbf{v}})(\gamma) = \sum_{y \rightarrow y' \in \mathcal{R}} k_{y \rightarrow y'} c^{v_{y \rightarrow y'}} (v_{y \rightarrow y'} * \gamma)(y' - y), \quad (6.2)$$

with $u * c w = \sum_i u_i w_i / c_i$.

Remark 6.3. If $\kappa \in \mathbb{R}_+^{\mathcal{R}}$ is not fixed then the function $f_{\kappa, \mathbf{v}}(c)$ can be seen as a polynomial function in the variables $k_{y \rightarrow y'}$ for all $y \rightarrow y' \in \mathcal{R}$.

A kinetic order for a general mass-action kinetics is intimately related to an influence specification for the network. If \mathbf{v} is a kinetic order for \mathcal{N} , define an influence specification $\mathcal{I}(\mathbf{v}) = (I_{y \rightarrow y'})_{y \rightarrow y' \in \mathcal{R}}$ by:

$$I_{y \rightarrow y'}(S_i) = \begin{cases} 1 & \text{if } i \in \text{supp}^+(v_{y \rightarrow y'}) \\ -1 & \text{if } i \in \text{supp}^-(v_{y \rightarrow y'}) \\ 0 & \text{otherwise.} \end{cases}$$

Reciprocally, if \mathcal{I} is an influence specification for \mathcal{N} , let $\mathbf{e}(\mathcal{I}) = (e_{y \rightarrow y'}(\mathcal{I}))_{y \rightarrow y' \in \mathcal{R}}$ be the kinetic order defined by

$$e_{y \rightarrow y'}(\mathcal{I}) = (I_{y \rightarrow y'}(S_1), \dots, I_{y \rightarrow y'}(S_n)).$$

Note that $\mathcal{I}(\mathbf{e}(\mathcal{I})) = \mathcal{I}$. If $\mathcal{I} = \mathcal{I}(\mathbf{v})$ then we say that \mathcal{I} is the influence specification associated to the kinetic order \mathbf{v} .

For an influence specification \mathcal{I} , let $\mathcal{K}_g(\mathcal{N}, \mathcal{I}) \subseteq \mathcal{K}(\mathcal{N}, \mathcal{I})$ be the set of general mass-action kinetics that are strictly monotonic with respect to \mathcal{I} . If \mathbf{v} is a kinetic order then it is straightforward to see that any kinetics $(\kappa, \mathbf{v}) \in \mathcal{K}_g(\mathcal{N})$ is differentiable with respect to the associated influence specification $\mathcal{I}(\mathbf{v})$. Therefore, $\mathcal{K}_g(\mathcal{N}, \mathcal{I}) \subseteq \mathcal{K}_d(\mathcal{N}, \mathcal{I})$. Likewise any kinetics $(\kappa, \mathbf{v}) \in \mathcal{K}_g(\mathcal{N})$ that is strictly monotonic with respect to an influence specification \mathcal{I} fulfills $\mathcal{I} = \mathcal{I}(\mathbf{v})$. Hence, we have the following lemma:

Lemma 6.4. *For any kinetic order \mathbf{v} we have $\mathcal{K}_g(\mathcal{N})[\mathbf{v}] \subseteq \mathcal{K}_g(\mathcal{N}, \mathcal{I}(\mathbf{v}))$. A kinetics (κ, \mathbf{v}) belongs to $\mathcal{K}_g(\mathcal{N}, \mathcal{I})$ if and only if $\mathcal{I}(\mathbf{v}) = \mathcal{I}$. Further*

$$\mathcal{K}_g(\mathcal{N}, \mathcal{I}) = \bigcup_{\mathbf{v} | \mathcal{I}(\mathbf{v}) = \mathcal{I}} \mathcal{K}_g(\mathcal{N})[\mathbf{v}].$$

Example 6.5. Consider the futile cycle of Example 3.2. The kinetic order $\mathbf{y} = (y_i)_{i=1,\dots,6}$ of any mass-action kinetics is (y_i denoting the vector corresponding to reaction r_i):

$$\begin{aligned} y_1 &= (1, 0, 1, 0, 0, 0), & y_2 &= (0, 0, 0, 0, 1, 0), & y_3 &= (0, 0, 0, 0, 1, 0), \\ y_4 &= (0, 1, 0, 1, 0, 0), & y_5 &= (0, 0, 0, 0, 0, 1), & y_6 &= (0, 0, 0, 0, 0, 1). \end{aligned}$$

Assume, for instance, that the concentration of the demodification enzyme S_2 acts as an enhancer or inhibitor of the modification of S_3 by the enzyme S_1 , that is, of reaction r_1 . This effect can be accounted for by considering a kinetic order $\mathbf{v} = (v_i)_{i=1,\dots,6}$ of the form

$$v_1 = (1, v, 1, 0, 0, 0), \quad v \in \mathbb{R} \setminus \{0\}, \quad v_i = y_i, \quad i > 1.$$

In this case, the species formation rate function $f_{\kappa, \mathbf{v}}$ is given by (denoting by k_i the rate constant of reaction r_i):

$$\begin{aligned} K_{S_1+S_3 \rightarrow S_5}(c) &= k_1 c_1 c_3 c_2^v & K_{S_5 \rightarrow S_1+S_3}(c) &= k_2 c_5 & K_{S_5 \rightarrow S_1+S_4}(c) &= k_3 c_5 \\ K_{S_2+S_4 \rightarrow S_6}(c) &= k_4 c_2 c_4 & K_{S_6 \rightarrow S_2+S_4}(c) &= k_5 c_6 & K_{S_6 \rightarrow S_2+S_3}(c) &= k_6 c_6. \end{aligned}$$

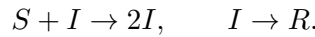
If for instance $v = 0.5$, then $K_{S_1+S_3 \rightarrow S_5}(c) = k_1 c_1 c_3 c_2^{0.5}$ while if $v = -0.5$, then $K_{S_1+S_3 \rightarrow S_5}(c) = k_1 c_1 c_3 c_2^{-0.5}$. The influence specification associated to \mathbf{v} , $\mathcal{I}(\mathbf{v})$, has the following non-zero signs (I_j refers to reaction r_j):

$$\begin{aligned} I_1(S_1) &= 1 & I_1(S_2) &= \text{sign}(v) & I_1(S_3) &= 1 & I_2(S_5) &= 1 & I_3(S_5) &= 1 \\ I_4(S_2) &= 1 & I_4(S_4) &= 1 & I_5(S_6) &= 1 & I_6(S_6) &= 1. \end{aligned}$$

The only possibly negative influence is $I_1(S_2)$. Note that $\mathcal{I}(\mathbf{v})$ depends only on the sign of v . In this example, the kinetic order $\mathbf{e}(\mathcal{I})$ is given by

$$\begin{aligned} e_1 &= (1, \text{sign}(v), 1, 0, 0, 0), & e_2 &= (0, 0, 0, 0, 1, 0), & e_3 &= (0, 0, 0, 0, 1, 0), \\ e_4 &= (0, 1, 0, 1, 0, 0), & e_5 &= (0, 0, 0, 0, 0, 1), & e_6 &= (0, 0, 0, 0, 0, 1). \end{aligned}$$

Example 6.6. So-called SIR (S=susceptible, I=infected, R=recovered) models are standard in epidemiology to describe the outbreak of an epidemics in a population. One particular SIR model¹ considers the network with set of species $\{S, I, R\}$ and reactions



The first reaction says that a susceptible individual might become infected in the presence of an infected. The second reaction says that infected individuals eventually recover. The SIR dynamics can be expressed in different ways. One possibility is the following set of differential equations²⁵:

$$\dot{c}_1 = -k_1 c_1' c_2, \quad \dot{c}_2 = k_1 c_1' c_2 - k_2 c_2, \quad \dot{c}_3 = k_2 c_2,$$

where c_1, c_2, c_3 are the concentrations of the species S, I, R , respectively, and $k_1, k_2 > 0$, $\nu \neq 0$ are the parameters of the model. That is, $K_{S+I \rightarrow 2I}(c) = k_1 c_1^\nu c_2$, and $K_{I \rightarrow R}(c) = k_2 c_2$. The amount $\bar{N} = S + I + R$ is conserved. All steady states have $c_2 = 0$ and $c_3 = \bar{N} - c_1$, which depends on the initial concentration of S . Hence, all infected individuals eventually recover. Further there are always multiple positive steady states in any stoichiometric class (given by \bar{N}).

The associated influence specification \mathcal{I} is given by

$$I_{S+I \rightarrow 2I}(I) = I_{I \rightarrow R}(I) = 1, \quad I_{S+I \rightarrow 2I}(S) = \text{sign}(\nu), \quad I_{S+I \rightarrow 2I}(R) = I_{I \rightarrow R}(S) = I_{I \rightarrow R}(R) = 0.$$

The parameter ν accounts for inhomogeneity in population mixing. If $\nu = 1$ then the population is homogenous and the disease spreads according to the law of mass-action, whereas if $\nu \neq 1$ then the population is inhomogenous and the kinetics is a general mass-action kinetics. If ν is negative then susceptible individuals are repelled by infected individuals. If we allow $\nu = 0$, then the spread of the disease would be independent of the number of infected individuals.

Many variants of the SIR model have been proposed,¹ not only at the level of the ODEs but also at the level of the interaction network. For example, individuals could be allowed to die, or recovered individuals could become susceptible again.

7 Injectivity for networks taken with general mass-action kinetics

The characterization of injectivity for networks with general mass-action kinetics will be key to provide a characterization of \mathcal{I} -injectivity for networks with general kinetics. In this section we provide criteria for a network to be injective with respect to different (nested) sets of general mass-action kinetics.

7.1 Fixed kinetic order

We first study kinetics with a given fixed kinetic order.

Theorem 7.1. *Let $\mathcal{N} = (\mathcal{S}, \mathcal{C}, \mathcal{R})$ be a network and \mathbf{v} a kinetic order. Then the following are equivalent:*

- (i) \mathcal{N} is injective over $\mathcal{K}_g(\mathcal{N})[\mathbf{v}]$.
- (ii) $\ker(J_c(f_{\kappa, \mathbf{v}})) \cap \Gamma = \{0\}$ for all $c \in \mathbb{R}_+^n$ and $\kappa \in \mathbb{R}_+^{\mathcal{R}}$.
- (iii) $\det(J_c(\tilde{f}_{\kappa, \mathbf{v}})) \neq 0$ for all $c \in \mathbb{R}_+^n$ and $\kappa \in \mathbb{R}_+^{\mathcal{R}}$.

To simplify the notation we introduce the following: for any set of m reactions, $R = \{y_1 \rightarrow y'_1, \dots, y_m \rightarrow y'_m\} \subseteq \mathcal{R}$, let

- $Z(R, \mathbf{v})$ be the $n \times m$ matrix whose i -th column is $v_{y_i \rightarrow y'_i}$
- $\Gamma(R)$ be the $n \times m$ matrix whose i -th column is $y'_i - y_i$
- For a set $J \subsetneq \{1, \dots, n\}$, let $Z(R, \mathbf{v})_J$ and $\Gamma(R)_J$ be equal the matrices $Z(R, \mathbf{v})$ and $\Gamma(R)$, respectively, with the rows $j \in J$ removed.

The next proposition is an adaptation of Ref. [8, Prop. 6.3, Cor. 7.3]. Let \mathcal{N} be a network and let $\mathcal{O}(\mathcal{N}) = \{i \mid S_i \rightarrow 0 \notin \mathcal{R}\}$ be the set of indices for which outflow reactions do not belong to \mathcal{R} . Finally, let $\mathcal{O}_d(\mathcal{N})$ be the set of subsets of $\mathcal{O}(\mathcal{N})$ of cardinality d and let $\mathbf{1} = (1, \dots, 1)$ denote the vector with 1 in each entry.

Proposition 7.2. *Let $\mathcal{N} = (\mathcal{S}, \mathcal{C}, \mathcal{R})$ be a network, \mathbf{v} a kinetic order, and s the dimension of the stoichiometric subspace. The determinant $\det(J_c(\tilde{f}_{\kappa, \mathbf{v}}))$ is a homogeneous polynomial in $\kappa = (k_{y \rightarrow y'})_{y \rightarrow y' \in \mathcal{R}}$ of total degree s and linear in each rate constant $k_{y \rightarrow y'}$.*

Further, let $R = \{y_1 \rightarrow y'_1, \dots, y_s \rightarrow y'_s\}$ be a set of s interactions from \mathcal{R} . The coefficient of the monomial $\prod_{i=1}^s k_{y_i \rightarrow y'_i}$ in $\det(J_c(\tilde{f}_{\kappa, \mathbf{v}}))$ for $c \in \mathbb{R}_+^n$ is

$$c^{-\mathbf{1} + \sum_{i=1}^s v_{y_i \rightarrow y'_i}} \sum_{J \in \mathcal{O}_d(\mathcal{N})} \det(Z(R, \mathbf{v})_J) \det(\Gamma(R)_J) \prod_{j \in J} c_j.$$

Remark 7.3. The determinant is in general *not* a polynomial in \mathbf{v} or in c because the coordinates of \mathbf{v} enter the expressions as exponents of c . However, the determinant $\det(Z(R, \mathbf{v})_J)$ is a polynomial function in the non-zero coordinates of $v_{y \rightarrow y'}$, $y \rightarrow y' \in R$, excluding the entries with indices in J .

Remark 7.4. Note that the expression in Proposition 7.2 differs by a sign $(-1)^s$ compared to the expression in Ref. [8, Cor. 7.3] where the coefficients are given in terms of $-\Gamma(R)_J$.

Proposition 7.5. *Let $\mathcal{N} = (\mathcal{S}, \mathcal{C}, \mathcal{R})$ be a network. The following are equivalent:*

- (i) \mathcal{N} is injective over $\mathcal{K}_g(\mathcal{N})[\mathbf{v}]$.
- (ii) *The non-zero products $\det(Z(R, \mathbf{v})_J) \det(\Gamma(R)_J)$ have the same sign for all sets R of s reactions from \mathcal{R} and $J \in \mathcal{O}_d(\mathcal{N})$. Further $\det(Z(R, \mathbf{v})_J) \det(\Gamma(R)_J) \neq 0$ for at least one choice of R and J .*

The matrix $Z(R, \mathbf{v})_J$ only relates to the influence specification \mathcal{I} on \mathcal{N} and not the structure of reactions. Similarly, the matrix $\Gamma(R)_J$ only relates to the structure of the reactions.

Example 7.6. Consider the futile example with the kinetic order \mathbf{v} introduced in Example 6.5. We have

$$\begin{aligned} \det(J_c(\tilde{f}_{\kappa, \mathbf{v}})) = & -c_1 c_2^v (c_2 + v c_3 + c_4) k_1 k_3 k_4 - c_1 c_2^v k_1 k_3 k_5 - c_1 c_2^v k_1 k_3 k_6 \\ & - c_2^{1+v} (c_1 + c_3) k_1 k_4 k_6 - c_2 k_2 k_4 k_6 - c_2 k_3 k_4 k_6. \end{aligned}$$

If $v \geq 0$, then all the terms of the determinant expansion have the same sign and by Theorem 7.1 the futile cycle is injective over $\mathcal{K}_g(\mathcal{N})[\mathbf{v}]$. If, on the contrary, $v < 0$, then the term $t = vc_1c_2^vc_3k_1k_3k_4$ has sign opposite to the rest of the terms. It follows that the futile cycle is not injective over $\mathcal{K}_g(\mathcal{N})[\mathbf{v}]$ if $v < 0$. The term t corresponds to the set of reactions $R = \{r_1, r_3, r_4\}$ and $J = \{1, 3, 6\}$. Indeed, for these sets we have

$$Z(R, \mathbf{v})_J = \begin{pmatrix} v & 0 & 1 \\ 0 & 0 & 1 \\ 0 & 1 & 0 \end{pmatrix}, \quad \Gamma(R)_J = \begin{pmatrix} 0 & 0 & -1 \\ 0 & 1 & -1 \\ 1 & -1 & 0 \end{pmatrix},$$

so that $\det(Z(R, \mathbf{v})_J) = -v$, $\det(\Gamma(R)_J) = 1$ and $c^{-1+\sum_{i=1}^s \mathbf{v}_i} \prod_{j \in J} c_j = c_1c_2^vc_3$. The sign of t depends on that of v , unless $v = 0$, in which case the term vanishes.

7.2 Kinetic orders with common influence specification

In the previous subsection we studied injectivity of a network over the set of kinetics $\mathcal{K}_g(\mathcal{N})[\mathbf{v}]$ for a fixed kinetic order \mathbf{v} . This family contains all general mass-action kinetics with kinetic order \mathbf{v} and arbitrary rate vector κ . The results therefore assert injectivity independently of the rate constants. In this subsection we are concerned with asserting injectivity for sets of general mass-action kinetics determined by influence specifications associated to kinetic orders. Specifically, we will characterize networks that are injective over any set of general mass-action kinetics for which the kinetic orders share common associated influence specification. Furthermore, we will use this characterization to provide a similar result for networks with associated influence specifications that are bounded by two given influence specifications.

To proceed we need some additional notation and definitions. We first introduce a partial order on the set of influence specifications for a network \mathcal{N} . One influence specification $\tilde{\mathcal{I}}$ is said to be smaller than another influence specification \mathcal{I} if $\tilde{\mathcal{I}}_{y \rightarrow y'}^+ \subseteq \mathcal{I}_{y \rightarrow y'}^+$ and $\tilde{\mathcal{I}}_{y \rightarrow y'}^- \subseteq \mathcal{I}_{y \rightarrow y'}^-$ for all $y \rightarrow y' \in \mathcal{R}$. If this is the case then we write $\tilde{\mathcal{I}} \preceq \mathcal{I}$. The minimal element in this order is the zero influence specification, that is the influence specification that takes 0 value for all reactions on all species. There is not a unique maximal element in this order but all maximal elements must fulfill $\mathcal{I}_{y \rightarrow y'}^+ \cup \mathcal{I}_{y \rightarrow y'}^- = \{1, \dots, n\}$.

For a $m \times k$ matrix V , let $\Sigma(V)$ be the *sign pattern* of V , that is, the $m \times k$ matrix indicating whether the entries of V are positive, negative or zero, respectively. If the entries with positive (resp. negative) signs of a matrix V' form a subset of the entries with positive (resp. negative) signs of V then we say that $\Sigma(V')$ is smaller than $\Sigma(V)$ and write $\Sigma(V') \preceq \Sigma(V)$. Consider the set of matrices

$$\Sigma_V = \{V', \text{ an } m \times k \text{ matrix} \mid \Sigma(V') = \Sigma(V)\}.$$

This set is called the *qualitative class* as V .²

Definition 7.7. An $m \times m$ square matrix V has a *signed-determinant* if the function

$$\delta := \text{sign} \circ \det : \Sigma_V \rightarrow \{-1, 0, 1\}, \quad V' \mapsto \text{sign}(\det(V'))$$

is constant. If the image of δ is not zero then V is called *sign-nonsingular* (SNS).

In other words, any matrix with the same sign pattern as a matrix V with signed-determinant has a determinant of the same sign as $\det(V)$. For example a 2×2 matrix with one negative entry and three positive is SNS, whereas one with all four entries positive is not. If one column of a matrix is identically zero, then the matrix has signed-determinant but is not SNS.

Any matrix V' in Σ_V is described by the specification of the absolute values $a_{i,j} = |v'_{i,j}|$ for each non-zero entry of V , such that $v'_{i,j} = \text{sign}(v_{i,j})a_{i,j}$. This gives a positive parameterization of Σ_V . The function $\det(\cdot)$ restricted to Σ_V becomes a polynomial function in the variables $a_{i,j}$ for $v_{i,j} \neq 0$, which we denote by $p_V(a_{i,j})$. In fact, if V is an $m \times m$ matrix, $p_V(a_{i,j})$ is either the zero polynomial or a homogeneous polynomial of degree m in $a_{i,j}$. Further, the degree of each monomial in each variable $a_{i,j}$ is either zero or one.

If V' is any matrix in Σ_V , then $\det(V') = p_V(|v'_{i,j}|)$. This equality remains valid for matrices V' such that $\Sigma(V') \preceq \Sigma(V)$, now with some variables $a_{i,j}$ vanishing.

Lemma 7.8. *Let V be an $m \times m$ square matrix. Then:*

- (i) *V has a signed-determinant if and only if p_V is either the zero polynomial or the non-zero coefficients of the monomials of p_V have sign $\delta(V)$.*
- (ii) *Let V' be an $m \times m$ matrix such that $\Sigma(V') \preceq \Sigma(V)$.*
 - (a) *If V has a signed-determinant then V' has a signed-determinant.*
 - (b) *If V is SNS and $\Sigma(V') = \Sigma(V)$ then V' is SNS.*
 - (c) *If V and V' are both SNS then $\delta(V') = \delta(V)$.*
 - (d) *If V' is SNS and V has a signed-determinant then V is SNS.*

Remark 7.9. If we consider a kinetic order $\mathbf{v} = (v_{y \rightarrow y'})_{y \rightarrow y' \in \mathcal{R}}$ to be a matrix with columns $v_{y \rightarrow y'}$ then the sign pattern is the same for all kinetic orders \mathbf{v}' with $\mathcal{I}(\mathbf{v}') = \mathcal{I}(\mathbf{v})$ and, further, $\Sigma(\mathbf{v}') \preceq \Sigma(\mathbf{v})$ if and only if $\mathcal{I}(\mathbf{v}') \preceq \mathcal{I}(\mathbf{v})$. We will be interested in the matrices $Z(R, \mathbf{v})_J$ for some sets R and J . The columns of $Z(R, \mathbf{v})_J$ agree with a subset of the columns of \mathbf{v} , hence

$$\Sigma(Z(R, \mathbf{v})_J) = \{Z(R, \mathbf{v}')_J \mid \mathcal{I}(\mathbf{v}') = \mathcal{I}(\mathbf{v})\}.$$

Also, $\Sigma(\mathbf{v}') \preceq \Sigma(\mathbf{v})$ implies that $\Sigma(Z(R, \mathbf{v}')) \preceq \Sigma(Z(R, \mathbf{v}))$.

Note that a network \mathcal{N} is injective over $\mathcal{K}_g(\mathcal{N}, \mathcal{I})$ if and only if \mathcal{N} is injective over $\mathcal{K}_g(\mathcal{N})[\mathbf{v}]$ for all kinetic orders with $\mathcal{I}(\mathbf{v}) = \mathcal{I}$ (Lemma 6.4). Hence, we can use Proposition 7.5 and Lemma 7.8 to derive a determinant criterion valid for $\mathcal{K}_g(\mathcal{N}, \mathcal{I})$. Similarly, if $\mathcal{I}_1, \mathcal{I}_2$ are influence specifications for \mathcal{N} such that $\mathcal{I}_1 \preceq \mathcal{I}_2$ then we can use Proposition 7.5 and Lemma 7.8 to derive a determinant criterion for the network to be injective over $\bigcup_{\mathcal{I} \mid \mathcal{I}_1 \preceq \mathcal{I} \preceq \mathcal{I}_2} \mathcal{K}_g(\mathcal{N}, \mathcal{I})$. Lemma 7.8 is applied to the matrices $V = Z(R, \mathbf{v})_J$ and $V' = Z(R, \mathbf{w})_J$ with kinetic orders \mathbf{v}, \mathbf{w} such that $\mathcal{I}(\mathbf{v}) = \mathcal{I}$ and $\mathcal{I}(\mathbf{w}) \preceq \mathcal{I}(\mathbf{v})$, respectively, in the two cases.

Proposition 7.10. *Let $\mathcal{N} = (\mathcal{S}, \mathcal{C}, \mathcal{R})$ be a network with influence specification \mathcal{I} and choose a kinetic order \mathbf{v} with $\mathcal{I}(\mathbf{v}) = \mathcal{I}$. Then \mathcal{N} is injective over $\mathcal{K}_g(\mathcal{N}, \mathcal{I})$ if and only if the following two statements hold:*

- (i) \mathcal{N} is injective over $\mathcal{K}_g(\mathcal{N})[\mathbf{v}]$.
- (ii) For all sets R of s reactions from \mathcal{R} and $J \in \mathcal{O}_d(\mathcal{N})$, if $\Gamma(R)_J$ is non-singular then $Z(R, \mathbf{v})_J$ has a signed-determinant.

In particular the proposition is true by choosing the kinetic order $\mathbf{v} = \mathbf{e}(\mathcal{I})$. The proposition provides a characterization of injectivity of a network \mathcal{N} over $\mathcal{K}_g(\mathcal{N}, \mathcal{I})$. The first condition guarantees that all coefficients in the polynomial expression of the determinant $\det(J_c(\tilde{f}_{\kappa, \mathbf{v}}))$ have the same sign or are zero, and that at least one coefficient is non-zero. Using Lemma 7.8(ii), the second condition ensures that this property is preserved for all kinetic orders \mathbf{v} with $\mathcal{I}(\mathbf{v}) = \mathcal{I}$.

Remark 7.11. Note that by virtue of Theorem 7.1 we have the following. If the determinant $\det(J_c(\tilde{f}_{\kappa, \mathbf{v}}))$ does not vanish for all $(\kappa, \mathbf{v}) \in \mathcal{K}_0 \subseteq \mathcal{K}_g(\mathcal{N}, \mathcal{I})$ then \mathcal{N} is injective over \mathcal{K}_0 , irrespectively whether any of the matrices $Z(R, \mathbf{v})_J$ has a signed-determinant. For example, if $\det(J_c(\tilde{f}_{\kappa, \mathbf{v}}))$ is not zero provided that $v_{y \rightarrow y', 1} > v_{y \rightarrow y', 2}$, then \mathcal{N} is injective over $\mathcal{K}_0 = \{K_g(\mathcal{N})[\mathbf{v}] \mid v_{y \rightarrow y', 1} > v_{y \rightarrow y', 2}, \mathcal{I}(\mathbf{v}) = \mathcal{I}\}$.

Proposition 7.12. *Let $\mathcal{N} = (\mathcal{S}, \mathcal{C}, \mathcal{R})$ be a network and $\mathcal{I}_1 \preceq \mathcal{I}_2$ two influence specifications for \mathcal{N} . Choose kinetic orders $\mathbf{v}_1, \mathbf{v}_2$ with $\mathcal{I}(\mathbf{v}_1) = \mathcal{I}_1$ and $\mathcal{I}(\mathbf{v}_2) = \mathcal{I}_2$.*

Then \mathcal{N} is injective over $\bigcup_{\mathcal{I}_1 \preceq \mathcal{I} \preceq \mathcal{I}_2} \mathcal{K}_g(\mathcal{N}, \mathcal{I})$ if and only if the following conditions hold:

- (i) \mathcal{N} is injective over $\mathcal{K}_g(\mathcal{N})[\mathbf{v}_1]$ and $\mathcal{K}_g(\mathcal{N})[\mathbf{v}_2]$.
- (ii) For all sets R of s reactions from \mathcal{R} and $J \in \mathcal{O}_d(\mathcal{N})$, if $\Gamma(R)_J$ is non-singular then $Z(R, \mathbf{v}_2)_J$ has a signed-determinant.

In particular the proposition is true for the kinetic orders $\mathbf{v}_1 = \mathbf{e}(\mathcal{I}_1)$ and $\mathbf{v}_2 = \mathbf{e}(\mathcal{I}_2)$. Proposition 7.12 provides a characterization of injectivity of a network \mathcal{N} over $\bigcup_{\mathcal{I}_1 \preceq \mathcal{I} \preceq \mathcal{I}_2} \mathcal{K}_g(\mathcal{N}, \mathcal{I})$. The second part of the first condition guarantees that all coefficients in the polynomial expression of the determinant $\det(J_c(\tilde{f}_{\kappa, \mathbf{v}_2}))$ have the same sign or are zero (Proposition 7.5). By Lemma 7.8(ii), the second condition ensures that this property is preserved for all kinetic orders \mathbf{w} with $\mathcal{I}(\mathbf{w}) \preceq \mathcal{I}_2$. The first part of the first condition ensures that at least one term is non-zero for all \mathbf{w} with $\mathcal{I}_1 \preceq \mathcal{I}(\mathbf{w})$. Thus, the first condition could be replaced by the condition that there is at least one non-zero term for some kinetics $(\kappa, \mathbf{v}_1) \in \mathcal{K}_g(\mathcal{N}, \mathcal{I}_1)$. Consequently, Proposition 7.12 can be rephrased to obtain:

Corollary 7.13. *Let $\mathcal{N} = (\mathcal{S}, \mathcal{C}, \mathcal{R})$ be a network and $\mathcal{I}_1, \mathcal{I}_2$ two influence specifications for \mathcal{N} such that $\mathcal{I}_1 \preceq \mathcal{I}_2$. The following statements are equivalent:*

(i) \mathcal{N} is injective over $\bigcup_{\mathcal{I}|\mathcal{I}_1 \preceq \mathcal{I} \preceq \mathcal{I}_2} \mathcal{K}_g(\mathcal{N}, \mathcal{I})$.

(ii) There is $\sigma \in \{0, 1\}$ such that $(-1)^\sigma \det(Z(R, \mathbf{v}_2)_J) \det(\Gamma(R)_J) \geq 0$ for all sets R of s reactions from \mathcal{R} , $J \in \mathcal{O}_d(\mathcal{N})$ and \mathbf{v}_2 with $\mathcal{I}(\mathbf{v}_2) = \mathcal{I}_2$. Further $\det(Z(R, \mathbf{v}_1)_J) \det(\Gamma(R)_J) \neq 0$ for at least one set R , some $J \in \mathcal{O}_d(\mathcal{N})$ and some \mathbf{v}_1 with $\mathcal{I}(\mathbf{v}_1) = \mathcal{I}_1$.

Propositions 7.10 and 7.12 provide the following corollary.

Corollary 7.14. Let $\mathcal{N} = (\mathcal{S}, \mathcal{C}, \mathcal{R})$ be a network and $\mathcal{I}_1 \preceq \mathcal{I}_2$ two influence specifications for \mathcal{N} . The network \mathcal{N} is injective over $\mathcal{K}_g(\mathcal{N}, \mathcal{I}_1)$ and $\mathcal{K}_g(\mathcal{N}, \mathcal{I}_2)$ if and only if \mathcal{N} is injective over $\bigcup_{\mathcal{I}|\mathcal{I}_1 \preceq \mathcal{I} \preceq \mathcal{I}_2} \mathcal{K}_g(\mathcal{N}, \mathcal{I})$.

A natural choice for the smaller influence specification \mathcal{I}_1 is in many contexts the complex dependent influence specification \mathcal{I}_C given by the kinetic order \mathbf{y} . Proposition 7.10 and the discussion above also imply the following corollary.

Corollary 7.15. Let \mathcal{N} be a network. Assume that \mathcal{N} is not injective over the set of mass-action kinetics $\mathcal{K}_g(\mathcal{N})[\mathbf{y}]$ and that the determinant $\det(J_c(\tilde{f}_{\kappa, \mathbf{y}}))$ is not identically zero. Then \mathcal{N} is not injective over $\mathcal{K}_g(\mathcal{N})[\mathbf{v}]$ for any kinetic order \mathbf{v} such that $\mathcal{I}_C \preceq \mathcal{I}(\mathbf{v})$.

Remark 7.16. Assume that $\mathcal{O}(\mathcal{N}) = \{1, \dots, n\}$, that is, the network \mathcal{N} has no outflow reactions. Then the determinants of $Z(R, \mathbf{v})_J$ and $\Gamma(R)_J$ for all reactions sets R and all subsets $J \subseteq \{1, \dots, n\}$ of cardinality s are exactly the $s \times s$ minors of $Z(\mathcal{R}, \mathbf{v})$ and $\Gamma(\mathcal{R})$, respectively.

Example 7.17. Consider the futile cycle with the kinetic order \mathbf{v} introduced in Example 6.5. The kinetic orders $\mathbf{w} = (w_i)_{i=1, \dots, 6}$ with $\mathcal{I}(\mathbf{w}) = \mathcal{I}(\mathbf{v})$ are of the form

$$\begin{aligned} w_1 &= (a_1, \text{sign}(v)w, a_2, 0, 0, 0), & w_2 &= (0, 0, 0, 0, a_3, 0), & w_3 &= (0, 0, 0, 0, a_4, 0), \\ w_4 &= (0, a_5, 0, a_6, 0, 0), & w_5 &= (0, 0, 0, 0, 0, a_7), & w_6 &= (0, 0, 0, 0, 0, a_8). \end{aligned}$$

with $w > 0$ and $a_i > 0$, $i = 1, \dots, 8$. The kinetic orders \mathbf{w} with $\mathcal{I}_C \preceq \mathcal{I}(\mathbf{w}) \preceq \mathcal{I}(\mathbf{v})$ also include the possibility $w = 0$. The network has no outflow reactions. Hence, using Remark 7.16, the products $\det(Z(R, \mathbf{w})_J) \det(\Gamma(R)_J)$ are the products of the corresponding 3×3 minors of $Z(\mathcal{R}, \mathbf{w})$ and $\Gamma(\mathcal{R})$. We have

$$Z(\mathcal{R}, \mathbf{w}) = \begin{pmatrix} a_1 & 0 & 0 & 0 & 0 & 0 \\ \text{sign}(v)w & 0 & 0 & a_5 & 0 & 0 \\ a_2 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & a_6 & 0 & 0 \\ 0 & a_3 & a_4 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & a_7 & a_8 \end{pmatrix} \quad \Gamma(\mathcal{R}) = \begin{pmatrix} -1 & 1 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & -1 & 1 & 1 \\ -1 & 1 & 0 & 0 & 0 & 1 \\ 0 & 0 & 1 & -1 & 1 & 0 \\ 1 & -1 & -1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & -1 & -1 \end{pmatrix}.$$

The products of the corresponding 3×3 minors of the two matrices are:

$$(0, -a_2a_4a_5, -\text{sign}(v)wa_4a_6, -a_2a_4a_6, -a_2a_4a_7, -a_2a_4a_8, -a_1a_6a_8, -a_2a_6a_8, -a_3a_6a_8, -a_4a_6a_8).$$

If $v \geq 0$ then the non-zero coefficients have negative sign. In that case it follows from Proposition 7.10 and Lemma 7.8(i) that the futile cycle is injective over $\mathcal{K}_g(\mathcal{N}, \mathcal{I}(\mathbf{v}))$ and in particular over $\mathcal{K}_g(\mathcal{N}, \mathcal{I}_C)$ (corresponding to $v = 0$). If $v < 0$ then the term $-\text{sign}(v)wa_4a_6$ is positive while the rest are negative and the futile cycle is not injective over $\mathcal{K}_g(\mathcal{N}, \mathcal{I}(\mathbf{v}))$.

Consider now the kinetic order \mathbf{w} with $w_i = y_i$ for $i \neq 1$ and $w_1 = (1, -w_{11}, 1, -w_{12}, 0, 0)$ with $w_{1i} > 0$. That is, we modify the kinetic order \mathbf{v} above to incorporate a negative influence of S_4 in the first reaction. Let $\mathcal{I} = \mathcal{I}(\mathbf{w})$. We repeat the procedure above and consider the family of kinetic orders with associated influence specification \mathcal{I} . The products of the corresponding 3×3 minors have the form:

$$(0, -a_2a_4a_5, -w_{12}a_4a_5 + w_{11}a_4a_6, -a_2a_4a_6, -a_2a_4a_7, -w_{12}a_4a_7, -a_2a_4a_8, -w_{12}a_4a_8, \\ -a_1a_6a_8, -a_2a_6a_8, -a_3a_6a_8, -a_4a_6a_8).$$

If a kinetic order \mathbf{w}' fulfills $\mathcal{I} = \mathcal{I}(\mathbf{w}')$ and $w_{12}a_5 > w_{11}a_6$, then all terms are negative and thus \mathcal{N} is injective over $\mathcal{K}_g(\mathcal{N})[\mathbf{w}']$ (Proposition 7.5, see also Remark 7.11). However, the term $-w_{12}a_4a_5 + w_{11}a_4a_6$ shows that one of the matrices $Z(R, \mathbf{w})_J$ does not have a signed-determinant (Lemma 7.8(i)). Therefore, \mathcal{N} is not injective over $\mathcal{K}_g(\mathcal{N}, \mathcal{I})$.

Example 7.18. Consider the futile cycle with the reaction dependent influence specification \mathcal{I}_R . Any kinetic order \mathbf{v} such that $\mathcal{I}(\mathbf{v}) = \mathcal{I}_R$ provides

$$Z(\mathcal{R}, \mathbf{v}) = \begin{pmatrix} v_1 & -v_4 & -v_7 & 0 & 0 & 0 \\ 0 & 0 & 0 & v_{10} & -v_{13} & -v_{16} \\ v_2 & -v_5 & 0 & 0 & 0 & -v_{17} \\ 0 & 0 & -v_8 & v_{11} & -v_{14} & 0 \\ -v_3 & v_6 & v_9 & 0 & 0 & 0 \\ 0 & 0 & 0 & -v_{12} & v_{15} & v_{18} \end{pmatrix}$$

with $v_i > 0$. Considering $\Gamma(\mathcal{R})$ as shown in the previous example, the non-zero products of corresponding 3×3 minors of these matrices have all negative sign and there is at least one that is non-negative. It follows that Proposition 7.10 holds and that the futile cycle is injective over $\mathcal{K}_g(\mathcal{N}, \mathcal{I}_R)$. The previous example showed that the futile cycle is injective over $\mathcal{K}_g(\mathcal{N}, \mathcal{I}_C)$. Hence, it follows from Corollary 7.14 that the cycle is injective over $\bigcup_{\mathcal{I}|\mathcal{I}_C \preceq \mathcal{I} \preceq \mathcal{I}_R} \mathcal{K}_g(\mathcal{N}, \mathcal{I})$.

The Mathematica code implementing the algorithm to decide whether the futile cycle is injective over $\mathcal{K}_g(\mathcal{N}, \mathcal{I}_R)$ is shown in Figure 3.

Remark 7.19. In general a network \mathcal{N} will not be injective over the set of all general mass-action kinetics $\mathcal{K}_g(\mathcal{N})$. In the case of the futile cycle (Example 7.6), the term $t = vc_1c_2^vc_3k_1k_3k_4$ is the only term depending on v . It changes sign with v whereas none of the other non-zero terms do. Hence, the futile cycle is not injective over $\mathcal{K}_g(\mathcal{N})$.

Define the stoichiometric matrix $\Gamma(\mathcal{R})$:

```
R = Transpose[{{-1,0,-1,0,1,0},{1,0,1,0,-1,0},{1,0,0,1,-1,0},
               {0,-1,0,-1,0,1},{0,1,0,1,0,-1},{0,1,1,0,0,-1}}];
{s,n} = {MatrixRank[R],Length[R[[1]]]};
```

Define the matrix $Z(\mathcal{R}, \mathbf{v})$:

```
Infl = Transpose[{{v[1],0,v[2],0,-v[3],0},{-v[4],0,-v[5],0,v[6],0},
                 {-v[7],0,0,-v[8],v[9],0},{0,v[10],0,v[11],0,-v[12]},
                 {0,-v[13],0,-v[14],0,v[15]},{0,-v[16],-v[17],0,0,v[18]}}];
lengthv = 18;
```

Compute the products of the corresponding minors (only non-repeated ones are kept):

```
{detZ,detG} = {Minors[Infl,s],Minors[R,s]};
coeffs = DeleteDuplicates[Flatten[detZ*detG]];
```

Check the signs of the coefficients:

```
Rules = {};
For[i=1,i<=lengthv,i++,AppendTo[Rules,v[i]->1]];
sign = DeleteCases[DeleteDuplicates[Sign[Flatten
    [MonomialList[coeffs]]/.Rules]],0];
If[Length[sign] == 1,
    Print["The network IS injective"],
    Print["The network is NOT injective"]; ];
```

Figure 3: Mathematica code implementing the algorithm to decide whether the futile cycle is injective over $\mathcal{K}_g(\mathcal{N}, \mathcal{I}_{\mathcal{R}})$.

7.3 Interaction networks defined by the set R of reactions

The conditions presented in the propositions in the previous sections relate to subnetworks of the network $\mathcal{N} = (\mathcal{S}, \mathcal{C}, \mathcal{R})$. Let R be a set of s reactions from \mathcal{R} and let $\mathcal{N}_R = (\mathcal{S}_R, \mathcal{C}_R, \mathcal{R}_R)$ be the network restricted to the reactions in R , that is: $\mathcal{S}_R = \mathcal{S}$, $\mathcal{C}_R = \{z \mid y \rightarrow y' \in R, z = y \text{ or } z = y'\}$ and $\mathcal{R}_R = R$. We say that \mathcal{N}_R is the restriction of \mathcal{N} to R . By definition there might be species of \mathcal{N}_R that are not involved in any reaction in \mathcal{R}_R .

Theorem 7.20. *Let $\mathcal{N} = (\mathcal{S}, \mathcal{C}, \mathcal{R})$ be a network, \mathbf{v} a kinetic order and let \mathcal{N}_R be the restriction of \mathcal{N} to a set R of s reactions from \mathcal{R} . Assume that the stoichiometric subspace of \mathcal{N}_R has dimension s and that \mathcal{N} is injective over $\mathcal{K}_g(\mathcal{N}, \mathbf{v})$. Let $\mathbf{v}_R = (v_{y \rightarrow y'})_{y \rightarrow y' \in R}$. Then either*

- (i) \mathcal{N}_R is injective over $\mathcal{K}_g(\mathcal{N}_R, \mathbf{v}_R)$ and all steady states are non-degenerate, or

(ii) \mathcal{N}_R has only degenerate steady states.

The theorem relates to Ref. [8, Cor. 8.1, Cor. 8.2], where injectivity of a network is studied relatively to injectivity of the network augmented with the “missing” outflow reactions. Also, the theorem relates to Ref. [14], where it is shown that if a restricted network \mathcal{N}_R with stoichiometric dimension s has multiple non-degenerate (positive) steady states then so does \mathcal{N} . However, Theorem 7.20 cannot be used to draw the same conclusion since non-injectivity does not imply that there are multiple steady states.

Example 7.21. Consider Example 4.9. The stoichiometric space has dimension one and there are two possible choices of reaction sets, $R_1 = \{S \rightarrow 2S\}$ and $R_2 = \{S \rightarrow 0\}$, both with empty index set, $J_1 = J_2 = \emptyset$. The networks \mathcal{N}_{R_1} and \mathcal{N}_{R_2} are both injective over $\mathcal{K}_g(\mathcal{N}_R, \mathbf{v}_R)$, but \mathcal{N} is not injective over $\mathcal{K}_g(\mathcal{N}, \mathbf{v})$.

8 Injectivity for strictly monotonic kinetics

In this section we extend the results on injectivity for general mass-action kinetics to cover \mathcal{I} -injectivity of a network \mathcal{N} over the set of strictly monotonic kinetics $\mathcal{K}(\mathcal{N}, \mathcal{I})$.

Theorem 8.1. *Let $\mathcal{N} = (\mathcal{S}, \mathcal{C}, \mathcal{R})$ be a network with influence specification \mathcal{I} . The following three statements are equivalent:*

- (i) \mathcal{N} is \mathcal{I} -injective over $\mathcal{K}(\mathcal{N}, \mathcal{I})$.
- (ii) \mathcal{N} is \mathcal{I} -injective over $\mathcal{K}_d(\mathcal{N}, \mathcal{I})$.
- (iii) \mathcal{N} is injective over $\mathcal{K}_g(\mathcal{N}, \mathcal{I})$.

The theorem implies that for a network to be \mathcal{I} -injective over $\mathcal{K}(\mathcal{N}, \mathcal{I})$ it is sufficient to be injective over \mathcal{K}_0 with $\mathcal{K}_g(\mathcal{N}, \mathcal{I}) \subseteq \mathcal{K}_0 \subseteq \mathcal{K}(\mathcal{N}, \mathcal{I})$. In Ref. [8, Prop. 5.2] it is shown that injectivity and \mathcal{I} -injectivity are equivalent notions for mass-action kinetics. Theorem 8.1 also implies that Ref. [8, Prop. 5.2] holds generally, namely that \mathcal{N} is \mathcal{I} -injective over $\mathcal{K}(\mathcal{N}, \mathcal{I})$ if and only if \mathcal{N} is injective over $\mathcal{K}(\mathcal{N}, \mathcal{I})$.

Theorem 8.2. *Let $\mathcal{N} = (\mathcal{S}, \mathcal{C}, \mathcal{R})$ be a network. Then the following are equivalent:*

- (i) $\ker(J_c(f_K)) \cap \Gamma = \{0\}$ for all $c \in \mathbb{R}_+^n$ and $K \in \mathcal{K}_d(\mathcal{N}, \mathcal{I})$.
- (ii) $\ker(J_c(f_{\kappa, \mathbf{v}})) \cap \Gamma = \{0\}$ for all $c \in \mathbb{R}_+^n$ and $(\kappa, \mathbf{v}) \in \mathcal{K}_g(\mathcal{N}, \mathcal{I})$.

If either of the two statements holds then \mathcal{N} is \mathcal{I} -injective over $\mathcal{K}(\mathcal{N}, \mathcal{I})$.

Recall from Proposition 5.4 that $\ker(J_c(f_K)) \cap \Gamma$ is zero if and only if $\det(J_c(\tilde{f}_K)) \neq 0$.

Corollary 8.3. *If \mathcal{N} is injective over $\mathcal{K}_d(\mathcal{N}, \mathcal{I})$ then \mathcal{N} cannot have positive degenerate steady states.*

The corollary follows immediately from Proposition 5.4. It also follows from Ref. [23] and from Ref. [8] for mass-action kinetics.

Example 8.4. According to Example 7.18 the futile cycle is injective over $\bigcup_{\mathcal{I}|\mathcal{I}_C \preceq \mathcal{I} \preceq \mathcal{I}_R} \mathcal{K}_g(\mathcal{N}, \mathcal{I})$. It follows from Theorem 8.1 that the futile cycle is \mathcal{I} -injective over $\mathcal{K}(\mathcal{N}, \mathcal{I})$ for any \mathcal{I} such that $\mathcal{I}_C \preceq \mathcal{I} \preceq \mathcal{I}_R$. That is, for any kinetics that is strictly monotonic with respect to an influence specification that is positive exactly for the species in the reactant complexes and may be negative for the species in the product complexes, the futile cycle is \mathcal{I} -injective and cannot admit multiple positive or non-overlapping steady states.

9 Extensions to other types of influence specifications

In Ref. [23] the authors introduce the term “weakly monotonic kinetics” which in some sense imposes a weaker requirement on the kinetics than the term “strictly monotonic kinetics” introduced here (Definition 4.6 and Lemma 4.8). In this section we assume that \mathcal{I} is an influence specification such that $\text{supp}(y) \subseteq I_{y \rightarrow y'}^+ \cup I_{y \rightarrow y'}^-$ (see also Remark 4.2).

Definition 9.1 (Ref. [23]). A kinetics $K = (K_{y \rightarrow y'})_{y \rightarrow y' \in \mathcal{R}}$ for a network $\mathcal{N} = (\mathcal{S}, \mathcal{C}, \mathcal{R})$ is *weakly monotonic with respect to an influence specification \mathcal{I}* if, for each pair of non-overlapping concentration vectors $a, b \in \Omega_K$, the following implications hold for all reactions $y \rightarrow y'$:

- (i) if $K_{y \rightarrow y'}(a) > K_{y \rightarrow y'}(b)$ then $\text{sign}(a_i - b_i) = I_{y \rightarrow y'}(S_i) \neq 0$ for some i .
- (ii) if $K_{y \rightarrow y'}(a) = K_{y \rightarrow y'}(b)$ then $a_i = b_i$ for all $i \in \text{supp}(y)$, or $\text{sign}(a_i - b_i) = I_{y \rightarrow y'}(S_i) \neq 0$ and $\text{sign}(a_j - b_j) = -I_{y \rightarrow y'}(S_j) \neq 0$ for some distinct i, j .

Let $\mathcal{K}_w(\mathcal{N}, \mathcal{I})$ denote the set of kinetics that are weakly monotonic with respect to \mathcal{I} .

Using the characterization of strictly monotonic kinetics provided in Lemma 4.8, we find that the two definitions differ in (ii), where it is required that $a_i = b_i$ for all $i \in I_{y \rightarrow y'}^+ \cup I_{y \rightarrow y'}^-$ and not just for $i \in \text{supp}(y)$. In this sense, Definition 9.1 imposes a weaker requirement on the kinetics than Definition 4.6 and

$$\mathcal{K}(\mathcal{N}, \mathcal{I}) \subset \mathcal{K}_w(\mathcal{N}, \mathcal{I}).$$

Note that in Ref. [23], an influence specification \mathcal{I} must fulfill $I_{y \rightarrow y'}(S_i) = 1$ for $i \in \text{supp}(y)$, which is not required in this paper. Definition 4.6 stipulates that all species play an equal role in the definition, whereas Definition 9.1 singles out the species in the reactant complex to have special importance. Below we show that our determinant criterion also applies to the broader definition of influence specification.

The determinant criterion derived in Corollary 7.13 can be adapted to derive a determinant criterion for a network \mathcal{N} to be \mathcal{I} -injective over $\mathcal{K}_w(\mathcal{N}, \mathcal{I})$. We first note that the influence specification given by $\tilde{I}_{y \rightarrow y'}(S_i) = I_{y \rightarrow y'}(S_i)$ for $i \in \text{supp}(y)$ and zero otherwise is a minimal element among all influence specifications for \mathcal{N} that are smaller than \mathcal{I} . Also note that if a, b are \mathcal{I} -non-overlapping then they are \mathcal{I}' -non-overlapping for all influence specifications \mathcal{I}' such that $\mathcal{I}' \preceq \mathcal{I}$. Hence, we have that

$$\mathcal{I}' \preceq \mathcal{I} \quad \text{implies} \quad \mathcal{K}_w(\mathcal{N}, \mathcal{I}') \subseteq \mathcal{K}_w(\mathcal{N}, \mathcal{I}). \quad (9.2)$$

Lemma 9.3. *If $K \in \mathcal{K}_w(\mathcal{N}, \mathcal{I})$ is a general mass-action kinetics then there is $\tilde{\mathcal{I}} \preceq \mathcal{I}' \preceq \mathcal{I}$ such that $K \in \mathcal{K}_g(\mathcal{N}, \mathcal{I}')$. That is,*

$$\mathcal{K}_w(\mathcal{N}, \mathcal{I}) \cap \mathcal{K}_g(\mathcal{N}) = \bigcup_{\mathcal{I}' | \tilde{\mathcal{I}} \preceq \mathcal{I}' \preceq \mathcal{I}} \mathcal{K}_g(\mathcal{N}, \mathcal{I}').$$

Theorem 9.4. *Let $\mathcal{N} = (\mathcal{S}, \mathcal{C}, \mathcal{R})$ be a network with influence specification \mathcal{I} . The following statements are equivalent:*

- (i) \mathcal{N} is \mathcal{I} -injective over $\mathcal{K}_w(\mathcal{N}, \mathcal{I})$.
- (ii) \mathcal{N} is \mathcal{I} -injective over $\bigcup_{\mathcal{I}' | \tilde{\mathcal{I}} \preceq \mathcal{I}' \preceq \mathcal{I}} \mathcal{K}(\mathcal{N}, \mathcal{I}')$.
- (iii) \mathcal{N} is injective over $\bigcup_{\mathcal{I}' | \tilde{\mathcal{I}} \preceq \mathcal{I}' \preceq \mathcal{I}} \mathcal{K}_g(\mathcal{N}, \mathcal{I}')$.

Together with Theorem 7.13 we derive a determinant criterion for a network \mathcal{N} to be \mathcal{I} -injective over $\mathcal{K}_w(\mathcal{N}, \mathcal{I})$. Further, it is straightforward to derive statements similar to those in Section 8 for $\mathcal{K}_w(\mathcal{N}, \mathcal{I})$.

Remark 9.5. In Ref. [23] the authors introduce the concepts of a *concordant network* and of a network being *concordant with respect to an influence specification \mathcal{I}* . To be concordant depends on the kinetics associated with the network only through the influence specification \mathcal{I} . Ref. [23] shows that to be concordant with respect to \mathcal{I} is equivalent to be injective over $\mathcal{K}_w(\mathcal{N}, \mathcal{I})$. Theorem 9.4 and Theorem 7.13 provide an equivalent characterization in terms of the influence specification through properties of the matrices $Z(R, \mathbf{v})_J$ and $\Gamma(R)_J$ and bring out an explicit relationship to the set of general mass-action kinetics.

10 The P -matrix property

In Ref. [2], a criterion is introduced that relates to injectivity of a network $\mathcal{N} = (\mathcal{S}, \mathcal{C}, \mathcal{R})$. The authors study a very broad class of kinetics termed *non-autocatalytic* (NAC) kinetics. In our terminology a NAC kinetics fulfills that (a) no species appear both in the reactant and the product complex of a reaction and (b) it belongs to $\bigcup_{\mathcal{I} \preceq \mathcal{I}_{\mathcal{R}}} \mathcal{K}(\mathcal{N}, \mathcal{I})$, where $\mathcal{I}_{\mathcal{R}}$ is the reaction

dependent influence specification. By (a), we have that $\mathcal{I}_{\mathcal{R}}$ is given by $\sigma_{y \rightarrow y'}(S_i) = \text{sign}(y_i - y'_i)$, $y \rightarrow y' \in \mathcal{R}$. We impose the same constraints in this section and consider kinetics such that (a) and (b) are fulfilled. This implies that the qualitative class of $-Z(R, \mathbf{v})$ is in the qualitative class of $\Gamma(R)$ for any $R \subseteq \mathcal{R}$ and $\mathcal{I}(\mathbf{v}) \preceq \mathcal{I}_{\mathcal{R}}$.

A square matrix is said to be a P -matrix if all principle minors of the matrix are positive. If the principle minors are non-negative the matrix is said to be a P_0 -matrix. Let K be a NAC kinetics for \mathcal{N} . The criterion in Ref. [2, Th. 4.1, Th. 4.3] states that

- (*) any square submatrix of the stoichiometric matrix $\Gamma(\mathcal{R})$ is SNS or singular if and only if $-J_c(f_K)$ (minus the Jacobian) is a P_0 -matrix.

Here $\Gamma(\mathcal{R})$ is the matrix with columns $y' - y$ for all $y \rightarrow y' \in \mathcal{R}$. If \mathcal{N} contains outflow reactions for all species $S_i \rightarrow 0$, $i = 1, \dots, n$, then (*) is equivalent to $-J_c(f_K)$ being a P -matrix (Ref. [2, Cor. 4.2, Th. 4.4]). Using the results in Ref. [9], it follows that \mathcal{N} is injective. If the criterion is fulfilled then the stoichiometric matrix is said to be *strongly sign determined* or SSD.

Thus, the criteria in Ref. [2] ensures injectivity when all outflow reactions are in \mathcal{R} . In particular, this implies that the stoichiometric subspace Γ has maximal dimension (equal to the number of species n in the network). If Γ does not have maximal dimension then $J_c(f_K)$ does not have maximal dimension either (equation (3.8)) and hence $-J_c(f_K)$ is not a P -matrix. Consequently, the criterion cannot be used to infer injectivity in this case. However, we show in the next theorem that the Jacobian of a *modified* version of \tilde{f}_K has full rank and is a P -matrix when K is a general mass-action kinetics. Let \hat{f}_K be \tilde{f}_K with the last s coordinates multiplied by -1 , that is

$$\hat{f}_K(c) = (\omega^1 \cdot c, \dots, \omega^d \cdot c, -f_{K,d+1}(c), \dots, -f_{K,n}(c)).$$

It follows that the Jacobian $J_c(\hat{f}_K)$ of \hat{f}_K is the Jacobian $J_c(\tilde{f}_K)$ with the bottom s rows multiplied by -1 .

Theorem 10.1. *Let $\mathcal{N} = (\mathcal{S}, \mathcal{C}, \mathcal{R})$ be a network and \mathcal{I} an influence specification. Let s be the dimension of the stoichiometric subspace and $d = n - s$. The following two statements are equivalent:*

- (i) \mathcal{N} is injective over $\mathcal{K}_g(\mathcal{N}, \mathcal{I})$.
- (ii) *There exist an order of the species in \mathcal{S} and a reduced basis of Γ^\perp such that the species S_{d+1}, \dots, S_n appear in some reactant complexes. Further, with the associated extended species formation rate function $\hat{f}_{\kappa, \mathbf{v}}$, the Jacobian $J_c(\hat{f}_{\kappa, \mathbf{v}})$ is a P -matrix for all kinetic orders \mathbf{v} with $\mathcal{I}(\mathbf{v}) = \mathcal{I}$ and $\kappa \in \mathbb{R}_+^{\mathcal{R}}$.*

We can replace $\mathcal{K}_g(\mathcal{N}, \mathcal{I})$ with $\bigcup_{\mathcal{I} \preceq \mathcal{I}_1 \preceq \mathcal{I}_2} \mathcal{K}_g(\mathcal{N}, \mathcal{I})$ and $\mathcal{I}(\mathbf{v}) = \mathcal{I}$ with $\mathcal{I}_1 \preceq \mathcal{I}(\mathbf{v}) \preceq \mathcal{I}_2$ in (i) and (ii), respectively, and the theorem remains valid. Under the NAC hypothesis, the criteria stated in Propositions 7.10 and 7.12 for arbitrary networks with general mass-action kinetics relate to the criterion (*):

Theorem 10.2. *Let $\mathcal{N} = (\mathcal{S}, \mathcal{C}, \mathcal{R})$ be a network and let $\mathcal{I}_{\mathcal{R}}$ be the reaction dependent influence specification. Assume that every square submatrix of $\Gamma(\mathcal{R})$ is SNS or singular. Let R be a set of s reactions from \mathcal{R} and $J \in \mathcal{O}_d(\mathcal{N})$, as in Proposition 7.2. Then:*

- (i) *If $\Gamma(R)_J$ is non-singular then $Z(R, \mathbf{v})_J$ has a signed-determinant for all \mathbf{v} with $\mathcal{I}(\mathbf{v}) \preceq \mathcal{I}_{\mathcal{R}}$.*
- (ii) *$(-1)^s \det(Z(R, \mathbf{v})_J) \det(\Gamma(R)_J) \geq 0$ for all \mathbf{v} with $\mathcal{I}(\mathbf{v}) \preceq \mathcal{I}_{\mathcal{R}}$.*

If one term in (ii) is positive for the kinetic order $\mathbf{e}(\mathcal{I}_{\mathcal{R}})$ then \mathcal{N} is injective over $\mathcal{K}_g(\mathcal{N}, \mathcal{I}_{\mathcal{R}})$ (Proposition 7.10). If one term is positive for the kinetic order \mathbf{y} then \mathcal{N} is in fact injective over $\cup_{\mathcal{I}|\mathcal{I}_{\mathcal{C}} \preceq \mathcal{I} \preceq \mathcal{I}_{\mathcal{R}}} \mathcal{K}_g(\mathcal{N}, \mathcal{I})$ (Proposition 7.12). In Ref. [2] positivity is guaranteed by imposing the network to contain all outflow reactions. However, if this is not the case then we are only guaranteed that the terms are non-negative and that the Jacobian of $\hat{f}_{\kappa, \mathbf{v}}$ is a P_0 -matrix.

In summary, we have shown that the criterion (*) in Ref. [2] implies our determinant criterion. Our criterion is *weaker* in that we only consider submatrices of $\Gamma(\mathcal{R})$ given by sets R and J , whereas Ref. [2] considers all square submatrices of $\Gamma(\mathcal{R})$. The additional requirement that one term is non-zero is necessary (and sufficient) to guarantee that \mathcal{N} is injective. In addition our approach ensures that if \mathcal{N} is injective then there cannot be degenerate steady states. Importantly, our results do not require the stoichiometric subspace to have maximal dimension but hold for arbitrary networks.

11 The interaction graph

An interaction network puts explicit constraints on the modeling equations of a dynamical system. We have used these constraints to derive criteria for the preclusion of multistationarity. However, conditions for the preclusion of multistationarity have also been given for generic dynamical systems described by ordinary differential equations and we will here review one condition that closely relates to our work.^{16, 24} This condition is also based on the Jacobian of the system and takes the form of a graphical condition. For this we need some preliminaries.

Let a dynamical system $\dot{c} = F(c) = (F_1(c), \dots, F_n(c))$ be given with $c = (c_1, \dots, c_n) \in \Omega \subseteq \mathbb{R}^n$, where Ω is a product of open intervals of \mathbb{R} and F_i is differentiable in the interior of Ω . The interaction graph $G(c)$ at c is the labeled directed graph with node set $\{1, \dots, n\}$ and labels in the set $\{-1, 1\}$ such that there is an edge from node j to node i if $\partial F_i(c)/\partial c_j \neq 0$. If this is the case, the edge has label given by $\text{sign}(\partial F_i(c)/\partial c_j)$.

A circuit in $G(c)$ is a sequence of distinct nodes i_1, \dots, i_m such that there is an edge (positive or negative) between i_k and i_{k+1} for all $k \leq m-1$ and one between i_m and i_1 . Two circuits are disjoint if they do not involve the same nodes. A k -nucleus is a collection of disjoint circuits which involves $1 \leq k \leq n$ nodes. The sign of a circuit is the product of the labels of the edges in the circuit. The sign of a k -nucleus is $(-1)^{p+1}$ where p is the number of circuits in the k -nucleus with sign equal to 1, i.e. positive.²⁴ A k -nucleus is variable if one edge in one of the circuits does not have constant sign in c .

In Ref. [16][Th. 2] (see below), a mild regularity condition is imposed on F . To keep the presentation clear, the reader is referred to the original paper for its description. We refer to it as condition (C). The following is true:

Theorem 11.1 (Ref. [16]). *Assume that the system $\dot{c} = F(c)$ has two non-degenerate steady states and that F fulfills condition (C). Then one of the following statements is true:*

- (i) *There exists $c \in \Omega$ such that $G(c)$ has two n -nuclei of different sign.*
- (ii) *There is a variable n -nucleus.*

The existence of non-degenerate steady states implies that the Jacobian of F is not singular for all c and that a n -nucleus exists for some c . If neither (i) nor (ii) above are fulfilled then the system cannot have multiple non-degenerate steady states. Note that in the statement there is no a priori restriction to pairs of *positive* steady states (unless Ω consists of positive vectors only). If (ii) is not fulfilled then preclusion of multiple steady states must follow from the failure of (i). Therefore, we assume that $G(c)$ does not depend on c , that is $G(c) = G$ for all c (and (ii) is not fulfilled by hypothesis).

Theorem 11.1 can then be rephrased as a statement about the preclusion of multiple non-degenerate steady states in any dynamical system with interaction graph G . We represent the interaction graph G in the form of a matrix $\{g_{j,i}\}_{j,i=1,\dots,n}$ such that $g_{j,i}$ is the label of the edge from node j to node i , being zero if such an edge does not exist.

In Theorem 11.3 below we show that failure of condition (i) in Theorem 11.1 for any such a graph is equivalent to a certain type of interaction networks not being injective over the class of differentiable kinetics. For that, we start by associating a collection of interaction networks with any labeled directed graph.

Definition 11.2. Let G be a labeled directed graph with node set $\{1, \dots, n\}$ and labels in the set $\{-1, 1\}$.

- Let $J = \{i \mid g_{j,i} = 0 \text{ for all } j\}$ be the set of nodes with no incoming edges. The network $\mathcal{N}_G = (\mathcal{S}, \mathcal{C}, \mathcal{R})$ consists of $\mathcal{S} = \{S_1, \dots, S_n\}$, $\mathcal{C} = \mathcal{S} \cup \{0\}$ and $\mathcal{R} = \{0 \rightarrow S_i, S_i \rightarrow 0 \mid i \notin J\}$.
- For $i \notin J$, let $H(i) = \{j \mid g_{j,i} \neq 0\}$. To every partition of $H(i)$ into two disjoint sets, $H(i) = H_1(i) \cup H_2(i)$, we associate an influence specification $\mathcal{I}_{H_1, H_2} = (I_{y \rightarrow y'})_{y \rightarrow y' \in \mathcal{R}}$ on \mathcal{N}_G by

$$I_{0 \rightarrow S_i}(S_j) = g_{j,i} \text{ if } j \in H_1(i), \quad I_{S_i \rightarrow 0}(S_j) = -g_{j,i} \text{ if } j \in H_2(i),$$

and zero otherwise.

If G is the (constant) interaction graph associated to a dynamical system as above then the set J corresponds to the set of indices for which F_i is constant. If J is not empty then the Jacobian of F is necessarily singular and Theorem 11.1 cannot be applied. By construction, the dimension s of the stoichiometric subspace of \mathcal{N}_G agrees with the cardinality of J .

Theorem 11.3. *Let G be a labeled directed graph with node set $\{1, \dots, n\}$ and labels in the set $\{-1, 1\}$. With the definitions of Definition 11.2 we have:*

(i) *For any $K \in \mathcal{K}_d(\mathcal{N}_G, \mathcal{I}_{H_1, H_2})$, the species formation rate function f_K has interaction graph G .*

Let s be the cardinality of J . The following two statements are equivalent:

(ii) *\mathcal{N}_G is \mathcal{I}_{H_1, H_2} -injective over $\mathcal{K}_d(\mathcal{N}_G, \mathcal{I}_{H_1, H_2})$.*

(iii) *G has at least one s -nucleus and all s -nuclei of G have the same sign.*

It follows that if (iii) is fulfilled then the network \mathcal{N}_G is injective over $\mathcal{K}_d(\mathcal{N}_G, \mathcal{I})$ for any influence specification \mathcal{I} obtained from any partition of $H(i)$, $i \notin J$.

Consider the case $s = n$. We have shown that if \mathcal{N}_G is \mathcal{I} -injective for any chosen influence specification \mathcal{I} , defined as in Definition 11.2, then any dynamical system $\dot{c} = F(c)$ with associated interaction graph G cannot have multiple non-degenerate steady states. Indeed, if \mathcal{N}_G is \mathcal{I} -injective then Theorem 11.3(iii) is fulfilled and consequently, Theorem 11.1(i) does not hold (assuming that F fulfills condition (C)).

If we chose the partition with $H_1(i) = H(i)$ and $H_2(i) = \emptyset$ then any kinetic order \mathbf{v} with $\mathcal{I}(\mathbf{v}) = \mathcal{I}$ is identically zero on the reactions $S_i \rightarrow 0$. Consequently, all $s \times s$ minors of $Z(R, \mathbf{v})$ vanish whenever R includes an outflow reaction $S_i \rightarrow 0$. Therefore, by Propositions 7.5, 7.10, and Theorem 8.1, \mathcal{N}_G is injective over $\mathcal{K}_d(\mathcal{N}_G, \mathcal{I})$ if and only if the network \mathcal{N}'_G , defined as the restriction of \mathcal{N}_G to the set of inflow reactions $S_i \rightarrow 0$, $i \notin J$, is injective over the induced influence specification \mathcal{I}' .

Therefore, we have proved the following corollary:

Corollary 11.4. *Let \mathcal{N} be the network consisting of n inflow reactions $0 \rightarrow S_i$ and let \mathcal{I} be an influence specification for \mathcal{N} . Further, let $\dot{c} = F(c)$ be any dynamical system in \mathbb{R}^n such that F fulfills condition (C) and has constant interaction graph $G = \{g_{j,i}\}$ with $g_{j,i} = I_{0 \rightarrow S_i}(S_j)$. If \mathcal{N} is injective over $\mathcal{K}_g(\mathcal{N}, \mathcal{I})$, then $\dot{c} = F(c)$ cannot have multiple non-degenerate steady states.*

Since the dimension of the stoichiometric space of \mathcal{N} in Corollary 11.4 is n , there is only one set of n reactions. By the definition of the influence specification, any kinetic order with associated influence \mathcal{I} has the signs in the columns of the matrix G . Therefore, \mathcal{N} is \mathcal{I} -injective over $\mathcal{K}_g(\mathcal{N}, \mathcal{I})$ if and only if the matrix associated with the interaction graph G is SNS. Therefore,

Corollary 11.5. *Let G be an $n \times n$ SNS matrix. Then, any dynamical system $\dot{c} = F(c)$ in \mathbb{R}^n that fulfills condition (C) and has constant interaction graph $G = \{g_{j,i}\}$ cannot have multiple non-degenerate steady states.*

Importantly, the corollary is saying that injectivity over the class of general mass-action kinetics suffices to preclude multistationarity in arbitrary dynamical systems, not only those originating from the specification of an interaction network and a kinetics. A possible extension to the theorem considers all influence specifications that are smaller than the one defined by G and, hence, it would assert injectivity with respect to $\mathcal{K}_w(\mathcal{N}, \mathcal{I})$.

Example 11.6. Consider Example 4.4. In Ref. [15] the gene network depicted in Figure 2 is modeled as

$$\dot{c}_1 = \frac{\alpha_1}{1 + \beta_1 c_3} - \delta_1 c_1, \quad \dot{c}_2 = \frac{\alpha_2 c_1}{1 + \beta_2 c_1} - \delta_2 c_2, \quad \dot{c}_3 = \frac{\alpha_3 c_1 c_2}{(1 + \beta_3 c_1)(1 + \beta_4 c_2)} - \delta_3 c_3,$$

for positive parameters, $\alpha_i, \beta_i, \delta_i$. Here degradation of each gene G_1, G_2, G_3 is incorporated. The interaction graph G associated with the system is constant with matrix

$$\begin{pmatrix} -1 & 1 & 1 \\ 0 & -1 & 1 \\ -1 & 0 & -1 \end{pmatrix}.$$

This matrix is SNS and therefore, by Corollary 11.5, the system cannot have multiple non-degenerate steady states.

Alternatively, the model arises as the dynamical system associated with the interaction network with reactions $0 \rightarrow G_i, G_i \rightarrow 0$, influence specification $\mathcal{I} = (I_{y \rightarrow y'})_{y \rightarrow y' \in \mathcal{R}}$ with (only non-zero values are listed)

$$I_{G_i \rightarrow 0}(G_i) = 1 \text{ for all } i, \quad I_{0 \rightarrow G_1}(G_3) = -1, \quad I_{0 \rightarrow G_2}(G_1) = I_{0 \rightarrow G_3}(G_1) = I_{0 \rightarrow G_3}(G_2) = 1,$$

and kinetics $K = (K_{y \rightarrow y'})_{y \rightarrow y' \in \mathcal{R}}$, with $K_{G_i \rightarrow 0} = \delta_i c_i$ and

$$K_{0 \rightarrow G_1}(c) = \frac{\alpha_1}{1 + \beta_1 c_3}, \quad K_{0 \rightarrow G_2}(c) = \frac{\alpha_2 c_1}{1 + \beta_2 c_1}, \quad K_{0 \rightarrow G_3}(c) = \frac{\alpha_3 c_1 c_2}{(1 + \beta_3 c_1)(1 + \beta_4 c_2)}.$$

We can use Proposition 7.10 and Theorem 8.1 to conclude that the interaction network is injective.

Note that in view of Corollary 11.4, the first procedure corresponds to analyze injectivity of the network with only inflow reactions and influence defined by the entries of G . Since a kinetics must be positive, the entry -1 in position $(1,1)$ of G corresponds to a decreasing kinetics in c_1 for the inflow reaction $0 \rightarrow G_1$. This is different from the system we started from, which had a negative summand $(-\delta_1 c_1)$. In other words, the dynamical systems characterized in Corollary 11.4 have positive species formation rate functions.

As we can deduce from the results above, preclusion of multistationarity by the methods of Ref. [16] is essentially preclusion of multistationarity in networks in which only inflow reactions are considered. Knowledge about the underlying network structure allows us to preclude multistationarity for a bigger class of dynamical systems. We do not only “see” the signs of the entries of the Jacobian, but also the terms that contribute to the signs. That is, if the network has a reaction of the form $A + B \rightarrow C$ then the species formation rate function has the term $K_{S_1+S_2 \rightarrow S_3}(c)$ in the entries 1, 2, 3 for any kinetics and it is negative for the first two. This is illustrated in the following simple example.

Example 11.7. Consider the interaction graph G with associated matrix $\begin{pmatrix} -1 & 1 \\ 1 & -1 \end{pmatrix}$. This matrix is not SNS and hence preclusion of multistationarity cannot be achieved by the methods in Ref. [16]. Consider now the network \mathcal{N} with set of reactions $\mathcal{R} = \{S_1 \rightarrow S_2, S_2 \rightarrow 0\}$. The dimension of the stoichiometric subspace is $n = 2$. For any kinetics K , the ODE system associated with the network is of the form

$$\dot{c}_1 = -K_{S_1 \rightarrow S_2}(c) \quad \dot{c}_2 = K_{S_1 \rightarrow S_2}(c) - K_{S_2 \rightarrow 0}(c).$$

Consider the influence specification with non-zero terms $I_{S_2 \rightarrow 0}(S_2) = 1$, $I_{S_1 \rightarrow S_2}(S_1) = 1$ and $I_{S_1 \rightarrow S_2}(S_2) = -1$. If $K \in \mathcal{K}_d(\mathcal{N}, \mathcal{I})$ then the Jacobian of the species formation rate function has interaction graph G . The only set of reactions of cardinality $n = 2$ is \mathcal{R} itself. For any kinetic order \mathbf{v} such that $\mathcal{I} = \mathcal{I}(\mathbf{v})$, the matrix $Z(\mathcal{R}, \mathbf{v})$ has sign 1 since the sign pattern is $\begin{pmatrix} 1 & 0 \\ -1 & 1 \end{pmatrix}$. Thus, it follows from Proposition 7.10 and Theorem 8.1 that \mathcal{N} is injective over $\mathcal{K}_d(\mathcal{N}, \mathcal{I})$ and multistationarity cannot occur.

12 Hill-type kinetics and injectivity

Let $\mathcal{N} = (\mathcal{S}, \mathcal{C}, \mathcal{R})$ be a network and \mathcal{I} an influence specification for \mathcal{N} . The key to the statements in Section 8 is that whenever there are two non-overlapping concentration vectors a, b such that $a - b \in \Gamma$ and $f_K(a) = f_K(b)$ for some kinetics $K \in \mathcal{K}(\mathcal{N}, \mathcal{I})$, then we can find two positive concentration vectors \tilde{a}, \tilde{b} such that $\tilde{a} - \tilde{b} \in \Gamma$ and $f_{\kappa, \mathbf{v}}(\tilde{a}) = f_{\kappa, \mathbf{v}}(\tilde{b})$ for some general mass-action kinetics $(\kappa, \mathbf{v}) \in \mathcal{K}_g(\mathcal{N}, \mathcal{I})$. However, the latter property could be fulfilled by many classes of kinetics other than the class of general mass-action kinetics.

One such class of kinetics is *Hill-type kinetics*. We say that a kinetics is of Hill-type with respect to an influence specification \mathcal{I} if $K = (K_{y \rightarrow y'})_{y \rightarrow y' \in \mathcal{R}}$ takes the form

$$K_{y \rightarrow y'}(c) = k_{y \rightarrow y'} \prod_{i=1}^n \frac{c_i^{v_{y \rightarrow y', i}}}{\delta_{y \rightarrow y', i} + c_i^{v_{y \rightarrow y', i}}},$$

with $c \in \overline{\mathbb{R}}_+^n$ (defined by continuity at the boundary), $k_{y \rightarrow y'} \in \mathbb{R}_+$, $\delta_{y \rightarrow y'} \in \overline{\mathbb{R}}_+^n$ and $v_{y \rightarrow y'} \in \mathbb{R}^n$ for all $y \rightarrow y' \in \mathcal{R}$, such that

$$\text{supp}^+(v_{y \rightarrow y'}) = I_{y \rightarrow y'}^+, \quad \text{supp}^-(v_{y \rightarrow y'}) = I_{y \rightarrow y'}^-, \quad \text{and} \quad \text{supp}(\delta_{y \rightarrow y'}) = \text{supp}(v_{y \rightarrow y'}).$$

The definition is very similar to that of general mass-action kinetics with the only difference being the factor $\delta_{y \rightarrow y', i}$ in the denominators. A term with $v_{y \rightarrow y', i} > 0$ defines a positive influence on the reaction $y \rightarrow y'$ while a term with $v_{y \rightarrow y', i} < 0$ defines a negative influence. Compared to general mass-action kinetics the constant $\delta_{y \rightarrow y', i}$ moderates a negative influence of a species S_i when it is in low concentration.

Let $\kappa = (k_{y \rightarrow y'})_{y \rightarrow y' \in \mathcal{R}}$, $\mathbf{d} = (\delta_{y \rightarrow y'})_{y \rightarrow y' \in \mathcal{R}}$, and $\mathbf{v} = (v_{y \rightarrow y'})_{y \rightarrow y' \in \mathcal{R}}$. We denote a Hill-type kinetics by $K = (\kappa, \mathbf{d}, \mathbf{v})$, the set of Hill-type kinetics for \mathcal{N} with respect to \mathcal{I} by $\mathcal{K}_H(\mathcal{N}, \mathcal{I})$ and the set of all Hill-type kinetics for \mathcal{N} by $\mathcal{K}_H(\mathcal{N})$. Hill-type kinetics include Michaelis-Menten kinetics as a special case when $v_{y \rightarrow y', i}$ is an integer.³ In contrast, general mass-action kinetics are not of Hill-type. However, general mass-action kinetics can be obtained as a limiting case of Hill-type kinetics by letting $k_{y \rightarrow y'}$ and the non-zero entries of $v_{y \rightarrow y'}$ tend to infinity such that $k_{y \rightarrow y'} / \prod_i v_{y \rightarrow y', i}$ converges to a positive constant.

Hill-type kinetics might be considered biochemically more reasonable than general mass-action kinetics as they are defined for all $\overline{\mathbb{R}}_+^n$ in contrast to general mass-action kinetics that might not be defined for points on the boundary of $\overline{\mathbb{R}}_+^n$. In addition, Hill-type kinetics or Michaelis-Menten kinetics are often obtained when variables (species) are eliminated from the modelling equations.³

Theorem 12.1. *Let $\mathcal{N} = (\mathcal{S}, \mathcal{C}, \mathcal{R})$ be a network and $a, b \in \mathbb{R}_+^n$. Then:*

- (i) *For every Hill-type kinetics $K = (\kappa, \mathbf{d}, \mathbf{v}) \in \mathcal{K}_H(\mathcal{N})$ there exists a general mass-action kinetics $(\lambda, \mathbf{w}) \in \mathcal{K}_g(\mathcal{N})$ such that $\Sigma(\mathbf{v}) = \Sigma(\mathbf{w})$, $f_K(a) = f_{\lambda, \mathbf{w}}(a)$ and $f_K(b) = f_{\lambda, \mathbf{w}}(b)$.*
- (ii) *For every general mass-action kinetics $(\lambda, \mathbf{w}) \in \mathcal{K}_g(\mathcal{N})$ there exists a Hill-type kinetics $K = (\kappa, \mathbf{d}, \mathbf{v}) \in \mathcal{K}_H(\mathcal{N})$ such that $\Sigma(\mathbf{v}) = \Sigma(\mathbf{w})$, $f_K(a) = f_{\lambda, \mathbf{w}}(a)$ and $f_K(b) = f_{\lambda, \mathbf{w}}(b)$.*

In particular, $f_{\lambda, \mathbf{w}}(a) = f_{\lambda, \mathbf{w}}(b)$ for some general mass-action kinetics $(\lambda, \mathbf{w}) \in \mathcal{K}_g(\mathcal{N})$ if and only if $f_K(a) = f_K(b)$ for some Hill-type kinetics $K = (\kappa, \mathbf{d}, \mathbf{v}) \in \mathcal{K}_H(\mathcal{N})$ such that $\Sigma(\mathbf{v}) = \Sigma(\mathbf{w})$.

As a consequence, injectivity of a network \mathcal{N} over $\mathcal{K}(\mathcal{N}, \mathcal{I})$ is guaranteed by injectivity of \mathcal{N} over $\mathcal{K}_H(\mathcal{N}, \mathcal{I})$. It also follows that \mathcal{N} is injective over $\mathcal{K}_g(\mathcal{N}, \mathcal{I})$ if and only if \mathcal{N} is injective over $\mathcal{K}_H(\mathcal{N}, \mathcal{I})$. Furthermore, we have that \mathcal{N} has multiple positive steady states in some stoichiometric class with respect to a Hill-type kinetics if and only if \mathcal{N} has multiple positive steady states in the same stoichiometric class with respect to a general mass-action kinetics.

A Proofs

Proof of Lemma 4.8. We first prove the forward implication, that is, that a strictly monotonic kinetics fulfills (i) and (ii). For two vectors u, v in \mathbb{R}^n and $i = 0, \dots, n$, let $u \oplus_i v$ denote the vector consisting of the first i coordinates of u and the last $n-i$ coordinates of v , with $u \oplus_0 v = v$ and $u \oplus_n v = u$. Assume that the kinetics is strictly monotonic with respect to \mathcal{I} . Let a, b be two non-overlapping vectors and consider $K_{y \rightarrow y'}(a)$ and $K_{y \rightarrow y'}(b)$ for some reaction $y \rightarrow y'$. We have

$$K_{y \rightarrow y'}(a) = K_{y \rightarrow y'}(b) + \sum_{i=1}^n K_{y \rightarrow y'}(b \oplus_{i-1} a) - K_{y \rightarrow y'}(b \oplus_i a). \quad (\text{A.1})$$

If $K_{y \rightarrow y'}(a) > K_{y \rightarrow y'}(b)$ then $K_{y \rightarrow y'}(b \oplus_{i-1} a) - K_{y \rightarrow y'}(b \oplus_i a) > 0$ for some i and in particular $K_{y \rightarrow y'}(b \oplus_{i-1} a) > 0$. Since K respects \mathcal{I} , it follows that $I_{y \rightarrow y'}^+ \subseteq \text{supp}(b \oplus_{i-1} a)$ and hence $b \oplus_{i-1} a$ belongs to $\Omega_K(I_{y \rightarrow y'}^+)$. The two vectors $b \oplus_{i-1} a$ and $b \oplus_i a$ differ only in the i -th entries, which are a_i, b_i , respectively. If also $b \oplus_i a$ belongs to $\Omega_K(I_{y \rightarrow y'}^+)$, then by Definition 4.6(iii) we have $i \in I_{y \rightarrow y'}^+ \cup I_{y \rightarrow y'}^-$. If $i \in I_{y \rightarrow y'}^+$, then $K_{y \rightarrow y'}$ is increasing in the i -th coordinate and hence $1 = \text{sign}(a_i - b_i) = I_{y \rightarrow y'}(S_i)$. If $i \in I_{y \rightarrow y'}^-$, then $K_{y \rightarrow y'}$ is decreasing in the i -th coordinate and $-1 = \text{sign}(a_i - b_i) = I_{y \rightarrow y'}(S_i)$. Hence (i) holds in this case. If $b \oplus_i a$ is not in $\Omega_K(I_{y \rightarrow y'}^+)$ then necessarily $b_i = 0$, $a_i > 0$ and $i \in I_{y \rightarrow y'}^+$. Hence $1 = \text{sign}(a_i) = I_{y \rightarrow y'}(S_i)$ and (i) holds as well.

Assume that $K_{y \rightarrow y'}(a) = K_{y \rightarrow y'}(b)$. Since a, b are non-overlapping, if $a_i = 0$ for some $i \in I_{y \rightarrow y'}^+$, then $b_i \neq 0$ for all $i \in I_{y \rightarrow y'}^+$. Since K respects the influence specification \mathcal{I} , we obtain that $K_{y \rightarrow y'}(a) = 0$ and $K_{y \rightarrow y'}(b) \neq 0$ contradicting the equality. Therefore, $a_i \neq 0$ and $b_i \neq 0$ for all $i \in I_{y \rightarrow y'}^+$, and hence $I_{y \rightarrow y'}^+ \subseteq \text{supp}(a)$, $I_{y \rightarrow y'}^+ \subseteq \text{supp}(b)$, and $I_{y \rightarrow y'}^+ \subseteq \text{supp}(b \oplus_i a)$ for all i . Consider the sum of differences in equation (A.1). If there is at least one positive and one negative term then we proceed as for $K_{y \rightarrow y'}(a) > K_{y \rightarrow y'}(b)$ and conclude that (ii) holds. Now assume that $K_{y \rightarrow y'}(b \oplus_{i-1} a) = K_{y \rightarrow y'}(b \oplus_i a)$ for all $i = 1, \dots, n$. In particular, taking $i \in I_{y \rightarrow y'}^+ \cup I_{y \rightarrow y'}^-$ it follows from Definition 4.6 that $a_i = b_i$ and (ii) holds. It completes the first part of the proof.

To prove the reverse implication, assume that (i) and (ii) are fulfilled. Let $c, d \in \Omega_K(I_{y \rightarrow y'}^+)$ be two vectors that differ only in the i -th coordinate. If $K_{y \rightarrow y'}(c) > K_{y \rightarrow y'}(d)$, then by (i) we have $\text{sign}(c_i - d_i) = I_{y \rightarrow y'}(S_i) \neq 0$. It follows that if $i \in I_{y \rightarrow y'}^0$, that is, $I_{y \rightarrow y'}(S_i) = 0$, then $K_{y \rightarrow y'}(\cdot)$ is constant in the i -th coordinate. If $i \in I_{y \rightarrow y'}^+ \cup I_{y \rightarrow y'}^-$ and $K_{y \rightarrow y'}(c) = K_{y \rightarrow y'}(d)$ then according to (ii) we have $c_i = d_i$ contradicting $c_i \neq d_i$ (the second option cannot occur since c and d differ in exactly one coordinate). Therefore $K_{y \rightarrow y'}(c) \neq K_{y \rightarrow y'}(d)$. Using (i) we conclude that $K_{y \rightarrow y'}(\cdot)$ is increasing/decreasing in the i -th coordinate depending on the sign of $I_{y \rightarrow y'}(S_i)$. This completes the proof. \square

Proof of Theorem 7.1. The equivalence of (ii) and (iii) is in Proposition 5.4. The equivalence of (i) and (ii) can be proved similarly to Theorem 5.6 in Ref. [8]: \mathcal{N} is not injective over $\mathcal{K}_g(\mathcal{N})[\mathbf{v}]$ if and only if there exists a rate vector κ and distinct $a, b \in \mathbb{R}_+^n$ such that $a - b \in \Gamma$

and $f_{\kappa, \mathbf{v}}(a) = f_{\kappa, \mathbf{v}}(b)$. Further, $\ker(J_c(f_{\eta, \mathbf{v}})) \cap \Gamma \neq \{0\}$ for some $c \in \mathbb{R}_+^n$ and a rate vector η if and only if there exists $\gamma \in \Gamma$ such that $J_c(f_{\eta, \mathbf{v}})(\gamma) = 0$. By definition and using equation (6.2),

$$\begin{aligned} f_{\kappa, \mathbf{v}}(a) - f_{\kappa, \mathbf{v}}(b) = 0 &\Leftrightarrow \sum_{y \rightarrow y' \in \mathcal{R}} k_{y \rightarrow y'} (a^{v_{y \rightarrow y'}} - b^{v_{y \rightarrow y'}})(y' - y) = 0, \\ J_c(f_{\eta, \mathbf{v}})(\gamma) = 0 &\Leftrightarrow \sum_{y \rightarrow y' \in \mathcal{R}} \eta_{y \rightarrow y'} c^{v_{y \rightarrow y'}} (v_{y \rightarrow y'} *_{\mathbf{c}} \gamma)(y' - y) = 0. \end{aligned}$$

It suffices to show that given distinct $a, b \in \mathbb{R}_+^n$ such that $\gamma := a - b \in \Gamma$ and some rate vector κ , there exist $c \in \mathbb{R}_+^n$ and $\eta \in \mathbb{R}_+^{\mathcal{R}}$ such that

$$k_{y \rightarrow y'} (a^{v_{y \rightarrow y'}} - b^{v_{y \rightarrow y'}}) = \eta_{y \rightarrow y'} c^{v_{y \rightarrow y'}} (v_{y \rightarrow y'} *_{\mathbf{c}} \gamma) \quad (\text{A.2})$$

and *vice versa*. Consider distinct $a, b \in \mathbb{R}_+^n$ such that $\gamma := a - b \in \Gamma$, $\gamma \neq 0$. If $a_i - b_i \neq 0$ define $c_i := \frac{a_i - b_i}{\log a_i / b_i} > 0$ and let $c_i = 1$ otherwise. Since $\gamma_i = 0$ if $a_i = b_i$, we have that $a^{v_{y \rightarrow y'}} = b^{v_{y \rightarrow y'}} e^{v_{y \rightarrow y'} *_{\mathbf{c}} \gamma}$. Note that the signs of $a^{v_{y \rightarrow y'}} - b^{v_{y \rightarrow y'}} = b^{v_{y \rightarrow y'}} (e^{v_{y \rightarrow y'} *_{\mathbf{c}} \gamma} - 1)$ and $v_{y \rightarrow y'} *_{\mathbf{c}} \gamma$ agree. If $v_{y \rightarrow y'} *_{\mathbf{c}} \gamma = 0$, let $\eta_{y \rightarrow y'} = 1$. Otherwise, we let $\eta_{y \rightarrow y'} = \frac{k_{y \rightarrow y'} (a^{v_{y \rightarrow y'}} - b^{v_{y \rightarrow y'}})}{c^{v_{y \rightarrow y'}} (v_{y \rightarrow y'} *_{\mathbf{c}} \gamma)} > 0$ and (A.2) is fulfilled. Reciprocally, given $\gamma \in \Gamma$, $\gamma \neq 0$, $c \in \mathbb{R}_+^n$ and $\eta \in \mathbb{R}_+^{\mathcal{R}}$, define $a, b \in \mathbb{R}_+^n$ by $b_i = a_i = 1$ if $\gamma_i = 0$ and $b_i = \gamma_i / (e^{\gamma_i / c_i} - 1) > 0$, $a_i = b_i e^{\gamma_i / c_i} > 0$ otherwise. Then $a - b = \gamma \in \Gamma$ and $a^{v_{y \rightarrow y'}} - b^{v_{y \rightarrow y'}} = b^{v_{y \rightarrow y'}} (e^{v_{y \rightarrow y'} *_{\mathbf{c}} \gamma} - 1)$ has the same sign as $y *_{\mathbf{c}} \gamma$. If $a^{v_{y \rightarrow y'}} - b^{v_{y \rightarrow y'}} = 0$, define $k_{y \rightarrow y'} = 1$. Otherwise, define $k_{y \rightarrow y'} = \frac{\eta_{y \rightarrow y'} (v_{y \rightarrow y'} *_{\mathbf{c}} \gamma)}{c^{v_{y \rightarrow y'}} (a^{v_{y \rightarrow y'}} - b^{v_{y \rightarrow y'}})} > 0$ and equality (A.2) is fulfilled. \square

Proof of Proposition 7.2. The Jacobian of $\tilde{f}_{\kappa, \mathbf{v}}$ at c is a matrix with entries being integers for the top d rows and of the form $k_{y \rightarrow y'} c^{\mathbf{m}}$ for some vector \mathbf{m} for the bottom s rows. It follows that $\det(J_c(\tilde{f}_{\kappa, \mathbf{v}}))$ is either zero or a homogeneous polynomial of degree s in the rate constants $k_{y \rightarrow y'}$.

Consider now an arbitrary network $\mathcal{N}' = (\mathcal{S}', \mathcal{C}', \mathcal{R}')$ with a general mass-action kinetics (λ, \mathbf{w}) and $\lambda = (l_{y \rightarrow y'})_{y \rightarrow y' \in \mathcal{R}'}$. The Jacobian of $f_{\lambda, \mathbf{w}}$ takes the form

$$J_c(f_{\lambda, \mathbf{w}})(\gamma) = \sum_{y \rightarrow y' \in \mathcal{R}} l_{y \rightarrow y'} c^{w_{y \rightarrow y'}} (w_{y \rightarrow y'} *_{\mathbf{c}} \gamma)(y' - y)$$

for $\gamma \in \mathbb{R}^n$. If $\{e_1, \dots, e_n\}$ denotes the canonical basis of \mathbb{R}^n , then the determinant of $J_c(f_{\lambda, \mathbf{w}})$ is

$$\det \left(\sum_{y \rightarrow y' \in \mathcal{R}} l_{y \rightarrow y'} c^{w_{y \rightarrow y'}} (w_{y \rightarrow y'} *_{\mathbf{c}} e_1)(y' - y), \dots, \sum_{y \rightarrow y' \in \mathcal{R}} l_{y \rightarrow y'} c^{w_{y \rightarrow y'}} (w_{y \rightarrow y'} *_{\mathbf{c}} e_n)(y' - y) \right).$$

By definition of the product $u *_c v$, it follows that $\det(J_c(f_{\lambda, \mathbf{w}}))$ is (recall that $\mathbf{1} = (1, \dots, 1)$)

$$c^{-1} \det \left(\sum_{y \rightarrow y' \in \mathcal{R}} l_{y \rightarrow y'} c^{w_{y \rightarrow y'}} (w_{y \rightarrow y'} \cdot e_1)(y' - y), \dots, \sum_{y \rightarrow y' \in \mathcal{R}} l_{y \rightarrow y'} c^{w_{y \rightarrow y'}} (w_{y \rightarrow y'} \cdot e_n)(y' - y) \right), \quad (\text{A.3})$$

where $u \cdot v$ is the standard scalar product in \mathbb{R}^n . Each term in the determinant expansion of (A.3) is a scalar times a product of the form

$$c^{-1} \prod_{i=1}^n l_{y_i \rightarrow y'_i} c^{w_{y_i \rightarrow y'_i}} = c^{-1 + \sum_{i=1}^n w_{y_i \rightarrow y'_i}} \prod_{i=1}^n l_{y_i \rightarrow y'_i}$$

for some set of n reactions $R = \{y_1 \rightarrow y'_1, \dots, y_n \rightarrow y'_n\} \subseteq \mathcal{R}$. Consider a fixed set R of n reactions and let \mathfrak{S}_n denote the set of all permutations of $\{1, \dots, n\}$. Then the coefficient of $c^{-1} \prod_{i=1}^n l_{y_i \rightarrow y'_i} c^{w_{y_i \rightarrow y'_i}}$ is

$$\begin{aligned} & \sum_{\sigma \in \mathfrak{S}_n} \det \left((w_{y_{\sigma(1)} \rightarrow y'_{\sigma(1)}} \cdot e_1)(y'_{\sigma(1)} - y_{\sigma(1)}), \dots, (w_{y_{\sigma(n)} \rightarrow y'_{\sigma(n)}} \cdot e_n)(y'_{\sigma(n)} - y_{\sigma(n)}) \right) \\ &= \sum_{\sigma \in \mathfrak{S}_n} \det \left(w_{y_{\sigma(1)} \rightarrow y'_{\sigma(1)}, 1}(y'_{\sigma(1)} - y_{\sigma(1)}), \dots, w_{y_{\sigma(n)} \rightarrow y'_{\sigma(n)}, n}(y'_{\sigma(n)} - y_{\sigma(n)}) \right) \\ &= \sum_{\sigma \in \mathfrak{S}_n} \left(\prod_{i=1}^n w_{y_{\sigma(i)} \rightarrow y'_{\sigma(i)}, i} \right) \det \left(y_{\sigma(1)} - y'_{\sigma(1)}, \dots, y'_{\sigma(n)} - y_{\sigma(n)} \right) \\ &= \left(\sum_{\sigma \in \mathfrak{S}_n} \left(\prod_{i=1}^n w_{y_{\sigma(i)} \rightarrow y'_{\sigma(i)}, i} \right) \text{sign}(\sigma) \right) \det(y'_1 - y_1, \dots, y'_n - y_n), \\ &= \det(w_{y_1 \rightarrow y'_1}, \dots, w_{y_n \rightarrow y'_n}) \det(y'_1 - y_1, \dots, y'_n - y_n) = \det(Z(R, \mathbf{w})) \det(\Gamma(R)), \end{aligned}$$

where $w_{y_{\sigma(i)} \rightarrow y'_{\sigma(i)}, i}$ is the i -th coordinate of $w_{y_{\sigma(i)} \rightarrow y'_{\sigma(i)}}$. Thus, the coefficient of the monomial $\prod_{i=1}^n l_{y_i \rightarrow y'_i}$ is

$$c^{-1 + \sum_{i=1}^n w_{y_i \rightarrow y'_i}} \det(Z(R, \mathbf{w})) \det(\Gamma(R)), \quad (\text{A.4})$$

Note that the argument used here is similar to the proof of Theorem 3.2 in Ref. [4]. Observe that if R contains repeated reactions, then $\det(\Gamma(R))$ is zero. Therefore $\det(J_c(f_{\lambda, \mathbf{w}}))$ is either zero or a homogeneous polynomial of degree n and linear in each $l_{y \rightarrow y'}$.

Let us go back to the network $\mathcal{N} = (\mathcal{S}, \mathcal{C}, \mathcal{R})$ with general mass-action kinetics (κ, \mathbf{v}) . Define a network $\mathcal{N}' = (\mathcal{S}', \mathcal{C}', \mathcal{R}')$ by augmenting \mathcal{N} with the reactions in $\mathcal{O}(\mathcal{N})$ such that $\mathcal{S}' = \mathcal{S}$, $\mathcal{C}' = \mathcal{C} \cup \{0\} \cup \{S_i \mid i \in \mathcal{O}(\mathcal{N})\}$ and $\mathcal{R}' = \mathcal{R} \cup \{S_i \rightarrow 0 \mid i \in \mathcal{O}(\mathcal{N})\}$. Let $w_{y \rightarrow y'} = v_{y \rightarrow y'}$ and $l_{y \rightarrow y'} = k_{y \rightarrow y'}$ if $y \rightarrow y' \in \mathcal{R}$, and $w_{S_i \rightarrow 0} = e_i$ and $l_{S_i \rightarrow 0} = 1$ if $i \in \mathcal{O}(\mathcal{N})$. Then, if $\lambda = (l_{y \rightarrow y'})_{y \rightarrow y' \in \mathcal{R}'}$, the pair (λ, \mathbf{w}) defines a general mass-action kinetics for \mathcal{N}' .

With this choice of λ we can view $\det(J_c(f_{\lambda, \mathbf{w}}))$ for \mathcal{N}' as a polynomial in $k_{y \rightarrow y'}$, linear in each of the rate constant (but which is no longer homogeneous because some rate constants are fixed to 1). It is shown in the Proof of Cor. 7.2 in Ref. [8] for mass-action kinetics that the non-zero terms in the polynomial expansion of $\det(J_c(\tilde{f}_{\kappa, \mathbf{v}}))$ precisely are the non-zero terms in the polynomial expansion of $\det(J_c(f_{\lambda, \mathbf{w}}))$ of degree s and that

$$(-1)^d \det(J_c(f_{\lambda, \mathbf{w}})) = \det(J_c(\tilde{f}_{\kappa, \mathbf{v}})) + \text{terms of total degree smaller than } s.$$

The proof does not make use of the specific form of the power-law $c^{v_{y \rightarrow y'}}$ and thus applies in this context as well. It follows that $\det(J_c(\tilde{f}_{\kappa, \mathbf{v}}))$ is a homogeneous polynomial in the rate constants $k_{y \rightarrow y'}$ of degree s and linear in each of them.

By definition of \mathcal{N}' , the terms of degree s in $\det(J_c(f_{\lambda, \mathbf{w}}))$ correspond to the sets \tilde{R} of n reactions in \mathcal{N}' such that contain exactly d outflow reactions $S_j \rightarrow 0$ for some $j \in \mathcal{O}(\mathcal{N})$. Let $R = \{y_1 \rightarrow y_1, \dots, y_s \rightarrow y'_s\}$ be the set of s reactions of \mathcal{N} in \tilde{R} and $J \in \mathcal{O}_d(\mathcal{N})$ be the set of the indices for the d outflow reactions. We can assume that the outflow reactions are the (ordered) reactions $s+1, \dots, n$ in \tilde{R} . Then the corresponding summand in $\det(J_c(\tilde{f}_{\kappa, \mathbf{v}}))$ is

$$\begin{aligned} (-1)^d \prod_{i=1}^s k_{y_i \rightarrow y'_i} c^{-1 + \sum_{i=1}^n w_{y_i \rightarrow y'_i}} \det(Z(\tilde{R}, \mathbf{w})) \det(\Gamma(\tilde{R})) \\ = \prod_{i=1}^s k_{y_i \rightarrow y'_i} c^{-1 + \sum_{i=1}^s v_{y_i \rightarrow y'_i}} \det(Z(R, \mathbf{v})_J) \det(\Gamma(R)_J) \prod_{j \in J} c_j, \end{aligned}$$

where we expanded $\det(Z(\tilde{R}, \mathbf{w}))$ and $\det(\Gamma(\tilde{R}))$ along the j -th columns for $j \in J$: each of the d reactions $S_j \rightarrow 0$ corresponds to a column in $\Gamma(\tilde{R})$ with zeros everywhere apart from the j -th entry which is -1 . Similarly for $Z(\tilde{R}, \mathbf{w})$ with the only difference that the entry is 1.

The coefficient of $\prod_{i=1}^s k_{y_i \rightarrow y'_i}$ is the sum of all coefficients obtained from sets \tilde{R} containing R and d outflow reactions. Therefore, the coefficient of $\prod_{i=1}^s k_{y_i \rightarrow y'_i}$ in $\det(J_c(\tilde{f}_{\kappa, \mathbf{v}}))$ is

$$c^{-1 + \sum_{i=1}^s v_{y_i \rightarrow y'_i}} \sum_{J \in \mathcal{O}_d(\mathcal{N})} \det(Z(R, \mathbf{v})_J) \det(\Gamma(R)_J) \prod_{j \in J} c_j.$$

This completes the proof. \square

Proof of Proposition 7.5. If (ii) holds then (i) is a consequence of Theorem 7.1 and Proposition 7.2. To show that (i) implies (ii) we do the following. We first show that for fixed $c \in \mathbb{R}_+^n$ the sum $A(R, c) = \sum_{J \in \mathcal{O}_d(\mathcal{N})} \det(Z(R, \mathbf{v})_J) \det(\Gamma(R)_J) \prod_{j \in J} c_j$ has the same sign for all sets R of s reactions from \mathcal{R} . If this is not the case we could choose rate vectors κ_1 and κ_2 such that the sign of the determinants of $J_c(f_{\kappa_1, \mathbf{v}})$ and $J_c(f_{\kappa_2, \mathbf{v}})$ would differ. This can be done because the determinant of $J_c(f_{\kappa, \mathbf{v}})$ is linear in each rate constant $k_{y \rightarrow y'}$ (Proposition 7.2). Hence, by

continuity of $\det(Z(R, \mathbf{v})_J)$, there would also be κ_0 such that the determinant of $J_c(f_{\kappa_0, \mathbf{v}})$ is zero, contradicting (i) and Theorem 7.1.

Secondly, if $\det(Z(R, \mathbf{v})_{J_i}) \det(\Gamma(R)_{J_i})$ differs in sign for two sets $J_1, J_2 \in \mathcal{O}_d(\mathcal{N})$ and fixed R , then we could choose concentration vectors a_1 and a_2 in \mathbb{R}_+^n such that $A(R, a_1)$ and $A(R, a_2)$, respectively, differ in sign. This is possible because $A(R, c)$ is linear in each coordinate c_i , $i = 1, \dots, n$. Hence, by continuity of $A(R, c)$, there is also $b \in \mathbb{R}_+^n$ such that $A(R, b) = 0$. Since $A(R, b) = 0$ and (i) holds by assumption then there is \tilde{R} such that $A(\tilde{R}, b) \neq 0$, say $A(\tilde{R}, b) > 0$. It follows that there is an open set $U_b \subseteq \mathbb{R}_+^n$ around b such that $A(R, \cdot)$ takes positive and negative values and $A(\tilde{R}, \cdot) > 0$. Taking $c \in U_b$, we reach a contradiction with the first part of the proof. \square

Proof of Lemma 7.8. (i) V has a signed-determinant if and only if the polynomial $p_V(a_{i,j})$ has a constant sign when evaluated in positive values of $a_{i,j}$. The equivalence follows from the fact that each variable has degree zero or one in $p_V(a_{i,j})$.

(ii) The determinant of V' is given by $p_V(|v'_{i,j}|)$. If V has a signed-determinant and $p_V \equiv 0$ then V is not SNS and $\text{sign}(\det(V'')) = 0$ for all V'' such that $\Sigma(V'') \preceq \Sigma(V)$. If p_V is not identically zero, then by (i) all non-zero monomials of p_V have the same sign. It follows that either $p_V(|v'_{i,j}|)$ vanishes or has sign $\delta(V)$. Any matrix V'' in the qualitative class of V' would fall in the same case. Statements (a)-(c) follow from this observation. If $p_V(|v'_{i,j}|)$ does not vanish, then $p_V(|v_{i,j}|) \neq 0$ as well and (d) follows. \square

Proof of Proposition 7.10. We will apply Lemma 7.8 to the matrices $Z(R, \mathbf{v})_J$. Recall that $\mathcal{I}(\mathbf{v}') = \mathcal{I}(\mathbf{v})$ if and only if $\Sigma(\mathbf{v}') = \Sigma(\mathbf{v})$. Assume that \mathcal{N} is injective over $\mathcal{K}_g(\mathcal{N}, \mathcal{I})$. Then (i) is trivially fulfilled. To see (ii), assume that $\det(\Gamma(R)_J) \neq 0$ for some R and J . Fix $V = Z(R, \mathbf{v})_J$ and let p_V be defined as in the discussion above Lemma 7.8. Let $\Sigma_{\mathbf{v}} = \{\mathbf{w} \mid \Sigma(\mathbf{w}) = \Sigma(\mathbf{v})\}$ such that the qualitative class of V is exactly the set of matrices $Z(R, \mathbf{w})_J$ for $\mathbf{w} \in \Sigma_{\mathbf{v}}$ (Remark 7.9). Let \mathbf{w}_J denote \mathbf{w} with the entries $j \in J$ for each $w_{y \rightarrow y'}$ removed. Then, $\det(Z(R, \mathbf{w})_J) = p_V(|\mathbf{w}_J|)$. We will show that $p_V(|\mathbf{w}_J|)$ has a constant sign for all $\mathbf{w} \in \Sigma_{\mathbf{v}}$. If $p_V(|\mathbf{w}_J|)$ does not have constant sign then p_V has at least a positive and a negative monomial. Since all degrees of the variables of p_V are zero or one, we can by continuity choose $\mathbf{v}_0 \in \Sigma_{\mathbf{v}}$ such that $p_V(|\mathbf{v}_{0,J}|) = 0$ (the set $\Sigma_{\mathbf{v}}$ inherits a Euclidean topology from the Euclidean space it is embedded in). Since \mathcal{N} is injective over $\mathcal{K}_g(\mathcal{N}, \mathcal{I})$, it is injective over $\mathcal{K}_g(\mathcal{N})[\mathbf{v}_0]$ and there must be some other sets R_0, J_0 with $\det(\Gamma(R_0)_{J_0}) \det(Z(R, \mathbf{v}_0)_{J_0}) \neq 0$, say $\det(Z(R_0, \mathbf{v}_0)_{J_0}) > 0$ (Proposition 7.5(ii)). In an open set Ω around $\mathbf{v}_0 \in \Sigma_{\mathbf{v}}$, $\det(Z(R, \cdot)_J)$ takes both positive and negative values. However Ω can be chosen such that $\det(Z(R_0, \tilde{\mathbf{v}})_{J_0}) > 0$ for all $\tilde{\mathbf{v}}$ in Ω . Hence $\det(Z(R_0, \tilde{\mathbf{v}})_{J_0}) \det(\Gamma(R_0)_{J_0})$ has a constant sign in Ω , while $\det(Z(R, \tilde{\mathbf{v}})_J) \det(\Gamma(R)_J)$ changes sign. We can therefore choose $\tilde{\mathbf{v}} \in \Omega$ for which \mathcal{N} is not injective over $\mathcal{K}_g(\mathcal{N})[\tilde{\mathbf{v}}]$ (Proposition 7.5(ii)) and we reach a contradiction. Hence (ii) is true.

To prove the reverse we do the following. Let \mathbf{w} be any kinetic order such that $\mathcal{I}(\mathbf{w}) = \mathcal{I}$. By (ii), whenever $\det(\Gamma(R)_J) \neq 0$ the matrix $Z(R, \mathbf{v})_J$ has a signed-determinant. Since $Z(R, \mathbf{w})_J$

belongs to the qualitative class of $Z(R, \mathbf{v})_J$, we have that

$$\text{sign}(\det(Z(R, \mathbf{w})_J) \det \Gamma(R)_J) = \text{sign}(\det(Z(R, \mathbf{v})_J) \det \Gamma(R)_J). \quad (\text{A.5})$$

By (i), \mathcal{N} is injective over $\mathcal{K}_g(\mathcal{N})[\mathbf{v}]$. Thus by Proposition 7.5(ii) all non-zero products $\det(Z(R, \mathbf{v})_J) \det \Gamma(R)_J$ have the same sign and at least one does not vanish. By (A.5) the same holds for \mathbf{w} and thus it follows from Proposition 7.5 that \mathcal{N} is injective over $\mathcal{K}_g(\mathcal{N})[\mathbf{w}]$ as well. \square

Proof of Proposition 7.12. If \mathcal{N} is injective over $\bigcup_{\mathcal{I}|\mathcal{I}_1 \preceq \mathcal{I} \preceq \mathcal{I}_2} \mathcal{K}_g(\mathcal{N}, \mathcal{I})$, then (i) is trivially fulfilled and (ii) follows from Proposition 7.10(ii).

Assume that (i) and (ii) hold. If \mathbf{v} is a kinetic order satisfying $\Sigma(\mathbf{v}) \preceq \Sigma(\mathbf{v}_2)$ then (ii) together with Remark 7.9 and Lemma 7.8(ii) imply that $Z(R, \mathbf{v})_J$ has a signed-determinant for all reaction sets R and $J \in \mathcal{O}_d(\mathcal{N})$ such that $\det(\Gamma(R)_J) \neq 0$. In particular, this statement holds for $\mathbf{v} = \mathbf{v}_1$. Further, assume that \mathbf{v} satisfies $\Sigma(\mathbf{v}_1) \preceq \Sigma(\mathbf{v}) \preceq \Sigma(\mathbf{v}_2)$. Since \mathcal{N} is injective over $\mathcal{K}_g(\mathcal{N})[\mathbf{v}_1]$, there exists at least one set of reactions R and $J \in \mathcal{O}_d(\mathcal{N})$ such that $\det(Z(R, \mathbf{v}_1)_J) \det(\Gamma(R)_J) \neq 0$. Since $Z(R, \mathbf{v}_1)_J$ has a signed-determinant, it must be SNS. From Lemma 7.8(ii,d) it follows that $Z(R, \mathbf{v})_J$ is also SNS and in particular that $\det(Z(R, \mathbf{v})_J) \det(\Gamma(R)_J) \neq 0$. Consequently, it follows from Proposition 7.10 that \mathcal{N} is injective over $\mathcal{K}_g(\mathcal{N}, \mathcal{I}(\mathbf{v}))$. Since $\bigcup_{\mathcal{I}|\mathcal{I}_1 \preceq \mathcal{I} \preceq \mathcal{I}_2} \mathcal{K}_g(\mathcal{N}, \mathcal{I}) = \bigcup_{\mathbf{v}|\Sigma(\mathbf{v}_1) \preceq \Sigma(\mathbf{v}) \preceq \Sigma(\mathbf{v}_2)} \mathcal{K}_g(\mathcal{N}, \mathcal{I}(\mathbf{v}))$, the proposition is proved. \square

Proof of Corollary 7.15. Since the determinant $\det(J_c(\tilde{f}_{\kappa, \mathbf{y}}))$ is not identically zero there is a term in its expansion in κ with positive coefficient and a term with negative coefficient. Since, $\mathcal{I}_c \preceq \mathcal{I}(v)$, the terms in the polynomial expansion of $\det(J_c(\tilde{f}_{\kappa, \mathbf{v}}))$ in κ cannot have all the same sign. Thus Proposition 7.5 implies that $\mathcal{K}_g(\mathcal{N})[\mathbf{v}]$ is not injective. \square

Proof of Theorem 7.20. Assume that \mathcal{N} is injective over $\mathcal{K}_g(\mathcal{N})[\mathbf{v}]$. Proposition 7.5(ii) implies that the non-zero products $\det(Z(R', \mathbf{v})_J) \det(\Gamma(R')_J)$ have the same sign δ for all sets R' of s reactions from \mathcal{R} and $J \in \mathcal{O}_d(\mathcal{N})$, and that at least one of the products is non-zero.

For the network \mathcal{N}_R there is only one set of s reactions from R , namely R itself (s is the dimension of the stoichiometric subspace of \mathcal{N}_R). Observe that $Z(R, \mathbf{v}_R) = Z(R, \mathbf{v})$ and that $\mathcal{O}(\mathcal{N}) \subseteq \mathcal{O}(\mathcal{N}_R)$, but that there might be outflow reactions in \mathcal{R} that are not in R . For $J \in \mathcal{O}_d(\mathcal{N})$, we have that $\det(Z(R, \mathbf{v}_R)_J) \det(\Gamma(R)_J)$ is either zero or has sign δ . If $J \notin \mathcal{O}_d(\mathcal{N})$ then there exists an outflow reaction $S_i \rightarrow 0$ that does not belong to R but belongs to \mathcal{R} . In this case $\det(\Gamma(R)_J) = \pm \det(\Gamma(\tilde{R})_{J \setminus \{i\}})$, where \tilde{R} is R augmented with the reaction $S_i \rightarrow 0$. Since the dimension of the stoichiometric subspace of \mathcal{N} is s and $S_i \rightarrow 0$ belongs to \mathcal{R} then $-S_i$ is a linear combination of the reaction vectors in R . Hence $\det(\Gamma(\tilde{R})_{J \setminus \{i\}}) = 0$. It follows that $\det(Z(R, \mathbf{v}_R)_J) \det(\Gamma(R)_J)$ is either zero or has sign δ for all $J \in \mathcal{O}_d(\mathcal{N}_R)$.

If $\det(Z(R, \mathbf{v}_R)_J) \det(\Gamma(R)_J) = 0$ for all $J \in \mathcal{O}_d(\mathcal{N}_R)$, then all steady states (if there are any) are degenerate (Proposition 5.4). If for some $J \in \mathcal{O}_d(\mathcal{N}_R)$ we have $\det(Z(R, \mathbf{v}_R)_J) \det(\Gamma(R)_J) \neq 0$, then it follows from Proposition 7.5 that \mathcal{N}_R is injective over $\mathcal{K}_g(\mathcal{N}_R)[\mathbf{v}_R]$ and hence all steady states are non-degenerate. \square

Proof of Theorem 8.1. The proof is similar in spirit to arguments presented in Ref. [23]. By Lemma 4.12, (i) implies (ii), and since general mass-action kinetics are differentiable with respect to the influence specification, (ii) implies (iii). Let us prove that (iii) implies (i). Assume that \mathcal{N} is injective over $\mathcal{K}_g(\mathcal{N}, \mathcal{I})$ but not \mathcal{I} -injective over $\mathcal{K}(\mathcal{N}, \mathcal{I})$. Then there exists $K \in \mathcal{K}(\mathcal{N}, \mathcal{I})$ and distinct non-overlapping vectors $a, b \in \mathbb{R}_+^n$ such that $\gamma := a - b \in \Gamma$ and $f_K(a) = f_K(b)$. Let $\alpha_{y \rightarrow y'} = K_{y \rightarrow y'}(a) - K_{y \rightarrow y'}(b)$ for $y \rightarrow y' \in \mathcal{R}$. Then,

$$\sum_{y \rightarrow y' \in \mathcal{R}} \alpha_{y \rightarrow y'}(y' - y) = \sum_{y \rightarrow y' \in \mathcal{R}} (K_{y \rightarrow y'}(a) - K_{y \rightarrow y'}(b))(y' - y) = 0. \quad (\text{A.6})$$

If $\alpha_{y \rightarrow y'} > 0$ for some reaction $y \rightarrow y' \in \mathcal{R}$, then by Lemma 4.8 we have that $\text{sign}(a_i - b_i) = I_{y \rightarrow y'}(S_i) \neq 0$ for some species S_i . Similarly, if $\alpha_{y \rightarrow y'} < 0$ then there is some species S_i such that $\text{sign}(a_i - b_i) = -I_{y \rightarrow y'}(S_i) \neq 0$. Finally, if $\alpha_{y \rightarrow y'} = 0$ then $\gamma_i = a_i - b_i = 0$ for all $i \in I_{y \rightarrow y'}^+ \cup I_{y \rightarrow y'}^-$ or there exist distinct indices i, j such that $\text{sign}(a_i - b_i) = I_{y \rightarrow y'}(S_i) \neq 0$ and $\text{sign}(a_j - b_j) = -I_{y \rightarrow y'}(S_j) \neq 0$ (here we use that a, b are non-overlapping.)

The above consideration implies that for each $y \rightarrow y' \in \mathcal{R}$ we can find vectors $v_{y \rightarrow y'} \in \mathbb{R}^n$ such that

$$\text{supp}^+(v_{y \rightarrow y'}) = I_{y \rightarrow y'}^+, \quad \text{supp}^-(v_{y \rightarrow y'}) = I_{y \rightarrow y'}^-, \quad \text{and} \quad \alpha_{y \rightarrow y'} = v_{y \rightarrow y'} \cdot \gamma. \quad (\text{A.7})$$

For example, assume that $\alpha_{y \rightarrow y'} > 0$ such that $\text{sign}(a_i - b_i) = I_{y \rightarrow y'}(S_i) \neq 0$ for some i . If $a_i > b_i$, choose $v_{y \rightarrow y', j}$ for $i \neq j$ such that $\text{sign}(v_{y \rightarrow y', j}) = I_{y \rightarrow y'}(S_j)$ and $\beta := \alpha_{y \rightarrow y'} - \sum_{j \neq i} v_{y \rightarrow y', j}(a_j - b_j) > 0$. By defining $v_{y \rightarrow y', i} = \beta/(a_i - b_i) > 0$, we obtain $\alpha_{y \rightarrow y'} = v_{y \rightarrow y'} \cdot \gamma$ and the above relations are satisfied. Similar arguments apply in the other situations.

For all real x we have that $\text{sign}(x) = \text{sign}(e^x - 1)$. If $v_{y \rightarrow y'} \cdot \gamma = 0$, let $\eta_{y \rightarrow y'} = 1$, and otherwise let $\eta_{y \rightarrow y'} = \alpha_{y \rightarrow y'}/(e^{v_{y \rightarrow y'} \cdot \gamma} - 1)$ such that

$$\alpha_{y \rightarrow y'} = v_{y \rightarrow y'} \cdot \gamma = \eta_{y \rightarrow y'}(e^{v_{y \rightarrow y'} \cdot \gamma} - 1)$$

for all $y \rightarrow y' \in \mathcal{R}$. Note that $\eta_{y \rightarrow y'} > 0$. Next we define two positive vectors $\tilde{a}, \tilde{b} \in \mathbb{R}_+^n$. For $i = 1, \dots, n$, if $\gamma_i \neq 0$, let \tilde{b}_i be the number fulfilling $\gamma_i = \tilde{b}_i(e^{\gamma_i} - 1)$. If $\gamma_i = 0$, let $\tilde{b}_i = 1$. Define $\tilde{a} = \tilde{b}e^\gamma$, then $\tilde{a} - \tilde{b} = \gamma \in \Gamma$ and $\tilde{a}/\tilde{b} = e^\gamma$. Note that both \tilde{a} and \tilde{b} are positive and from the definitions we obtain

$$\alpha_{y \rightarrow y'} = \frac{\eta_{y \rightarrow y'}}{\tilde{b}^{v_{y \rightarrow y'}}}(\tilde{a}^{v_{y \rightarrow y'}} - \tilde{b}^{v_{y \rightarrow y'}}).$$

Define $\kappa = (k_{y \rightarrow y'})_{y \rightarrow y' \in \mathcal{R}}$ by $k_{y \rightarrow y'} = \eta_{y \rightarrow y'}/\tilde{b}^{v_{y \rightarrow y'}}$ and let $\mathbf{v} = (v_{y \rightarrow y'})_{y \rightarrow y' \in \mathcal{R}}$. Then $(\kappa, \mathbf{v}) \in \mathcal{K}_g(\mathcal{N}, \mathcal{I})$ and from (A.6)

$$f_{\kappa, \mathbf{v}}(\tilde{a}) = \sum_{y \rightarrow y' \in \mathcal{R}} k_{y \rightarrow y'} \tilde{a}^{v_{y \rightarrow y'}}(y' - y) = \sum_{y \rightarrow y' \in \mathcal{R}} k_{y \rightarrow y'} \tilde{b}^{v_{y \rightarrow y'}}(y' - y) = f_{\kappa, \mathbf{v}}(\tilde{b}).$$

Consequently \mathcal{N} is not injective over $\mathcal{K}_g(\mathcal{N}, \mathcal{I})$. \square

Proof of Theorem 8.2. Clearly (i) implies (ii). Assume that (ii) holds and $\ker(J_c(f_K)) \cap \Gamma \neq \{0\}$ for some $c \in \mathbb{R}_+^n$ and $K \in \mathcal{K}_d(\mathcal{N}, \mathcal{I})$. Then there exists $c^* \in \mathbb{R}_+^n$ and $\gamma \in \Gamma$ such that $J_{c^*}(f_K)(\gamma) = 0$. By equation (3.8) and letting $v_{y \rightarrow y'}$ be the partial derivative of $K_{y \rightarrow y'}$ evaluated at c^* , we have

$$J_{c^*}(f_K)(\gamma) = \sum_{y \rightarrow y' \in \mathcal{R}} \left(\frac{\partial K_{y \rightarrow y'}}{\partial c}(c^*) \cdot \gamma \right) (y' - y) = \sum_{y \rightarrow y' \in \mathcal{R}} (v_{y \rightarrow y'} \cdot \gamma)(y' - y) = 0.$$

If we let $\kappa = (k_{y \rightarrow y'})_{y \rightarrow y' \in \mathcal{R}}$ with $k_{y \rightarrow y'} = 1$ for all $y \rightarrow y' \in \mathcal{R}$ and $c = (1, \dots, 1) \in \mathbb{R}_+^n$, we have

$$J_c(f_K)(\gamma) = \sum_{y \rightarrow y' \in \mathcal{R}} k_{y \rightarrow y'} c^{v_{y \rightarrow y'}} (v_{y \rightarrow y'} * c \cdot \gamma)(y' - y) = 0.$$

Since the kinetics K belongs to $\mathcal{K}_d(\mathcal{N}, \mathcal{I})$, Definition 4.11 implies that $\text{supp}^+(v_{y \rightarrow y'}) = I_{y \rightarrow y'}^+$ and $\text{supp}^-(v_{y \rightarrow y'}) = I_{y \rightarrow y'}^-$. It follows that $\mathbf{v} = \{v_{y \rightarrow y'}\}_{y \rightarrow y' \in \mathcal{R}}$ is a kinetic order with $\mathcal{I}(\mathbf{v}) = \mathcal{I}$ and $(\kappa, \mathbf{v}) \in \mathcal{K}_g(\mathcal{N}, \mathcal{I})$. Further $J_c(f_{\kappa, \mathbf{v}})(\gamma) = J_{c^*}(f_K)(\gamma) = 0$ contradicting (ii). \square

Proof of Lemma 9.3. Assume that $K \in \mathcal{K}_w(\mathcal{N}, \mathcal{I})$ is a general mass-action kinetics with kinetic order \mathbf{v} . Then $K \in \mathcal{K}_g(\mathcal{N}, \mathcal{I}')$ with $\mathcal{I}' = \mathcal{I}(\mathbf{v})$. Let us prove that $\mathcal{I}' \preceq \mathcal{I}$. Let i be such that $I'_{y \rightarrow y'}(S_i) = 1$, that is, $v_{y \rightarrow y', i} > 0$. Consider $a, b \in \mathbb{R}_+^n$ such that $a_j = b_j$, $i \neq j$, and $a_i > b_i$. Then $K_{y \rightarrow y'}(a) > K_{y \rightarrow y'}(b)$ because K is a general mass-action kinetics. By Definition 9.1(i) and using that a, b only differ in the index i , we have $I_{y \rightarrow y'}(S_i) = \text{sign}(a_i - b_i) = 1$. Therefore, $I'_{y \rightarrow y'}(S_i) = I_{y \rightarrow y'}(S_i)$. We proceed similarly if $I'_{y \rightarrow y'}(S_i) = -1$ to conclude that $\mathcal{I}' \preceq \mathcal{I}$. \square

Proof of Theorem 9.4. By Lemma 9.3, (i) implies (iii). (ii) and (iii) are equivalent due to Theorem 8.1. That (iii) implies (i) can be proved similarly to the proof of Theorem 8.1. The only difference lies in choosing $v_{y \rightarrow y'} \in \mathbb{R}^n$ for each $y \rightarrow y' \in \mathcal{R}$, see equation (A.7). Now we choose $v_{y \rightarrow y'} \in \mathbb{R}^n$ such that $\text{supp}(y) \subseteq \text{supp}(v_{y \rightarrow y'})$, and

$$\text{supp}^+(v_{y \rightarrow y'}) \subseteq I_{y \rightarrow y'}^+, \quad \text{supp}^-(v_{y \rightarrow y'}) \subseteq I_{y \rightarrow y'}^-, \quad \text{and} \quad \alpha_{y \rightarrow y'} = v_{y \rightarrow y'} \cdot \gamma.$$

This can be done using an argument similar to the argument provided below equation (A.7). \square

Proof of Theorem 10.1. The proof follows the idea given in Ref. [8][Sect. 10]. By Proposition 7.5 and Theorem 7.1, \mathcal{N} is injective over $\mathcal{K}_g(\mathcal{N})[\mathbf{v}]$ if and only if $\det(J_c(\tilde{f}_{\kappa, \mathbf{v}})) \neq 0$ for all $c \in \mathbb{R}_+^n$, which happens if and only if

(**) there exists $\sigma \in \{0, 1\}$ such that $(-1)^\sigma \det(Z(R, \mathbf{v})_J) \det(\Gamma(R)_J) \geq 0$ for all sets R of s reactions from \mathcal{R} and $J \in \mathcal{O}_d(\mathcal{N})$ and at least one of these terms is not zero.

Assume that $(**)$ holds. Let S_{i_1}, \dots, S_{i_m} be the species of \mathcal{N} that are not in reactant complexes (a complex y in a reaction $y \rightarrow y'$). Then the lower s rows of $J_c(\tilde{f}_{\kappa, \mathbf{v}})$ are zero in entries i_1, \dots, i_m . If $(**)$ holds then $\det(J_c(\tilde{f}_{\kappa, \mathbf{v}})) \neq 0$ and the columns i_1, \dots, i_m of $J_c(\tilde{f}_{\kappa, \mathbf{v}})$ are linearly independent. Since the lower $s \times m$ submatrix is identically zero, it follows that the upper $d \times m$ matrix has rank m (and in particular $m \leq d$). As a consequence, we can reorder the species in \mathcal{S} such that S_{i_1}, \dots, S_{i_m} are the first m species and we are guaranteed that there exists a reduced basis of Γ^\perp with that order. Note that this order is independent of the kinetic order \mathbf{v} .

Consider now the order given above. Because $m \leq d$, the species S_{d+1}, \dots, S_n of \mathcal{N} appear in some reactant complex and the NAC assumption guarantees that the diagonal entries of $J_c(\tilde{f}_{\kappa, \mathbf{v}})$ in the rows $d+1, \dots, n$ are non-zero and negative. Since the diagonal entries in rows $1, \dots, d$ are all equal to one, it follows that the product of the diagonal entries is a term in the expansion of $\det(J_c(\hat{f}_{\kappa, \mathbf{v}}))$ and has sign 1 (by construction of \hat{f}). The matrix $J_c(\hat{f}_{\kappa, \mathbf{v}})$ has full rank and is a P -matrix: if \mathcal{N} fulfills $(**)$ then all non-zero terms in the determinant expansion of $J_c(\hat{f}_{\kappa, \mathbf{v}})$ have sign 1. Each principle minor can be obtained as a sum of terms in the determinant expansion of $J_c(\hat{f}_{\kappa, \mathbf{v}})$ divided by the (positive) diagonal entries of the rows not taken in the minor. Since this sum contains at least the non-zero diagonal product term, all principle minors are positive. Consequently, $J_c(\hat{f}_{\kappa, \mathbf{v}})$ is a P -matrix.

Therefore, if \mathcal{N} is injective over $\mathcal{K}_g(\mathcal{N}, \mathcal{I})$, then $(**)$ holds for all kinetic orders \mathbf{v} such that $\mathcal{I}(\mathbf{v}) = \mathcal{I}$ and consequently there exists an order of the species of \mathcal{N} such that the matrices $J_c(\hat{f}_{\kappa, \mathbf{v}})$ are P -matrices. Reciprocally, if $J_c(\hat{f}_{\kappa, \mathbf{v}})$ is a P -matrix for all \mathbf{v} then, using the results in Ref. [9], we conclude that $\hat{f}_{\kappa, \mathbf{v}}$ is an injective function for all \mathbf{v} and hence that \mathcal{N} is injective over $\mathcal{K}_g(\mathcal{N}, \mathcal{I})$. \square

Proof of Theorem 10.2. Consider a set of reactions R , an index set J and the kinetic order $\mathbf{e} = \mathbf{e}(\mathcal{I}_R)$ given by $e_{y \rightarrow y', i} = \text{sign}(y_i - y'_i)$. Since every square submatrix of $\Gamma(\mathcal{R})$ is SNS or singular then it is in particular true for $\Gamma(R)_J$ for any R and J . The matrix $Z(R, \mathbf{e})_J$ belongs to the same qualitative class as $-\Gamma(R)_J$. Thus, if $\Gamma(R)_J$ is non-singular, it is SNS and so is $-\Gamma(R)_J$. It follows from Lemma 7.8(ii) and Remark 7.9 that $Z(\mathcal{R}, \mathbf{w})_J$ has a signed-determinant for all \mathbf{w} such that $\mathcal{I}(\mathbf{w}) \preceq \mathcal{I}_R$. Further, the sign of $\det(Z(R, \mathbf{w})_J)$ either agrees with the sign of $\det(-\Gamma(R)_J) = \det(Z(R, \mathbf{e})_J)$ or is zero. Because $\Gamma(R)_J$ is an $s \times s$ matrix, we have $(-1)^s \det(\Gamma(R)_J) = \det(-\Gamma(R)_J)$, and we obtain $(-1)^s \det(Z(R, \mathbf{w})_J) \det(\Gamma(R)_J) \geq 0$ for all \mathbf{w} such that $\mathcal{I}(\mathbf{w}) \preceq \mathcal{I}_R$. \square

Proof of Theorem 11.3. (i) For shortness write $\mathcal{I} = \mathcal{I}_{H_1, H_2}$ and $\mathcal{N} = \mathcal{N}_G$. Let $K \in \mathcal{K}_d(\mathcal{N}, \mathcal{I})$. The species formation rate function f_K has entries $f_{K, i} = 0$ if $i \in J$ and $f_{K, i}(c) = K_{0 \rightarrow S_i}(c) - K_{S_i \rightarrow 0}(c)$ if $i \notin J$. If $i \in J$, then $\partial f_{K, i}(c) / \partial c_j = 0 = g_{j, i}$ as desired. Assume that $i \notin J$. Since K is differentiable with respect to \mathcal{I} , we have that $\text{sign}(\frac{\partial K_{0 \rightarrow S_i}(c)}{\partial c_j}) = I_{0 \rightarrow S_i}(S_j)$ and

$\text{sign}(\frac{\partial K_{S_i \rightarrow 0}(c)}{\partial c_j}) = I_{S_i \rightarrow 0}(S_j)$. By definition of \mathcal{I} ,

$$\frac{\partial f_{K,i}(c)}{\partial c_j} = \frac{\partial K_{0 \rightarrow S_i}(c)}{\partial c_j} - \frac{\partial K_{S_i \rightarrow 0}(c)}{\partial c_j} = \begin{cases} 0 & j \notin H(i) \\ \frac{\partial K_{0 \rightarrow S_i}(c)}{\partial c_j} & j \in H_1(i) \\ -\frac{\partial K_{S_i \rightarrow 0}(c)}{\partial c_j} & j \in H_2(i). \end{cases}$$

In each case, the sign is $g_{j,i}$. It follows that the species formation rate function has interaction graph G .

We proceed to prove the equivalence between (ii) and (iii). The network \mathcal{N} is \mathcal{I} -injective over $\mathcal{K}_d(\mathcal{N}, \mathcal{I})$ if and only if \mathcal{N} is injective over $\mathcal{K}_g(\mathcal{N}, \mathcal{I})$ (Theorem 8.1). Hence we prove the equivalence between (ii) for $\mathcal{K}_g(\mathcal{N}, \mathcal{I})$ and (iii).

Recall the criteria for injectivity in Proposition 7.10. Let the dimension of the stoichiometric subspace of \mathcal{N} be s and note that there are s reactions $S_i \rightarrow 0$, $i \notin J$. Consequently, $\mathcal{O}_d(\mathcal{N}) = \{J\}$. Consider the interaction graph G . Nodes in the set J have no incoming edges, hence a node $i \in J$ cannot be part of any circuit of G . Consequently, any s -nucleus of G contains precisely the s nodes in $\{1, \dots, n\} \setminus J$. Furthermore, an s -nucleus of G is an s -nucleus of the matrix G with the rows and columns with index in J removed, and vice versa. Denote this $s \times s$ matrix by G_J .

Consider first the case where $H_2(i) = \emptyset$ for all i . Let \mathbf{v} be a kinetic order such that $\mathcal{I}(\mathbf{v}) = \mathcal{I}$ and consider $Z(R, \mathbf{v})_J$ for the set $R = \{0 \rightarrow S_i | i \notin J\}$ (ordered according to i). We have that $\Gamma(R)_J$ is non-singular and, by construction, the sign pattern of $Z(R, \mathbf{v})_J$ equals the sign pattern of G_J . Consequently, an s -nucleus of G_J is an s -nucleus of $Z(R, \mathbf{v})_J$ and vice versa. An s -nucleus corresponds to a non-zero term in the expansion of $\det(Z(R, \mathbf{v})_J)$.²⁴ Let a be such a term and $N(a)$ the corresponding s -nucleus. By Ref. [24], Lemma 1, we have

$$\text{sign}(N(a)) = \text{sign}(a)(-1)^{s+1}.$$

Hence, all s -nucleus of G have the same sign if and only if all non-zero terms in the expansion of $\det(Z(R, \mathbf{v})_J)$ have the same sign. Further, there is an s -nucleus if and only if there is a non-zero term. It remains to remark that had we chosen any other set R of s reactions from \mathcal{R} then there would be a zero column in $Z(R, \mathbf{v})_J$ ($I_{S_i \rightarrow 0}(S_j) = 0$ for all j) and hence $\det(Z(R, \mathbf{v})_J) = 0$. This proves the equivalence between (ii) and (iii) when $H_2(i) = \emptyset$ for all i .

Denote the interaction network above by $(\mathcal{N}_0, \mathcal{I}_0)$, $Z(R, \mathbf{v})_J$ by Z_0 and $\Gamma(R)_J$ by Γ_0 . Assume that $H_2(j)$ is non-empty for some indices j . Any set R of s reactions such that $\Gamma(R)_J$ is non-singular consists of exactly one reaction among $0 \rightarrow S_i$ and $S_i \rightarrow 0$ for every $i \notin J$. We can assume that the reactions are ordered according to the species index i and let i_1, \dots, i_τ be the indices for which outflow reactions are in R . Then $\det(\Gamma(R)_J) = (-1)^\tau \det(\Gamma_0)$. For this set, if we change the sign of the columns i_1, \dots, i_τ of $\det(Z(R, \mathbf{v})_J)$, the resulting matrix has a sign pattern smaller in the order \preceq than that of G_J , i.e., of Z_0 . Hence $\det(Z(R, \mathbf{v})_J)$ is

either zero or has the same sign as $(-1)^\tau \det(Z_0)$. Hence, $\text{sign}(\det(Z(R, \mathbf{v})_J) \det(\Gamma(R)_J)) = \text{sign}(\det(Z_0) \det(\Gamma_0))$ or zero.

Take a non-zero term in the polynomial expansion of $\det(Z_0)$. It is of the form $\mu = \pm \prod_{i \notin J} v_{0 \rightarrow S_i, \sigma(i)}$ with σ a permutation on the set of indices satisfying $i \notin J$. Build a set R consisting of reactions $0 \rightarrow S_i$ if $\sigma(i) \in H_1(i)$ and $S_i \rightarrow 0$ if $\sigma(i) \in H_2(i)$. Then, $(-1)^\tau \mu$ is a term in the polynomial expansion of $\det(Z(R, \mathbf{v})_J)$. This guarantees the existence of a set of reactions for which $\det(Z(R, \mathbf{v})_J) \det(\Gamma(R)_J) \neq 0$. Consequently, \mathcal{N} is injective over $\mathcal{K}_g(\mathcal{N}, \mathcal{I})$ if and only if \mathcal{N}_0 is injective over $\mathcal{K}_g(\mathcal{N}_0, \mathcal{I}_0)$ and the equivalence between (ii) and (iii) follows from the case above. \square

Proof of Theorem 12.1. Let $a, b \in \mathbb{R}_+^n$, $(\lambda, \mathbf{w}) \in \mathcal{K}_g(\mathcal{N})$ and $\mathcal{I} = \mathcal{I}(\mathbf{w})$. For each $y \rightarrow y' \in \mathcal{R}$, define $\delta_{y \rightarrow y', i} = 0$ and $v_{y \rightarrow y', i} = 0$ for all $i \in I_{y \rightarrow y'}^0$ (that is, for all i such that $w_{y \rightarrow y', i} = 0$). Let $M_{y \rightarrow y'} \in \mathbb{R}_+$ be such that $M_{y \rightarrow y'} a_i^{w_{y \rightarrow y', i}} < 1$ and $M_{y \rightarrow y'} b_i^{w_{y \rightarrow y', i}} < 1$ for all $i \in I_{y \rightarrow y'}^+ \cup I_{y \rightarrow y'}^-$. Then we can find $\delta_{y \rightarrow y', i} \in \overline{\mathbb{R}}_+$ and $v_{y \rightarrow y', i} \in \mathbb{R}$ such that,

$$\frac{a_i^{v_{y \rightarrow y', i}}}{\delta_{y \rightarrow y', i} + a_i^{v_{y \rightarrow y', i}}} = M_{y \rightarrow y'} a_i^{w_{y \rightarrow y', i}} \quad \text{and} \quad \frac{b_i^{v_{y \rightarrow y', i}}}{\delta_{y \rightarrow y', i} + b_i^{v_{y \rightarrow y', i}}} = M_{y \rightarrow y'} b_i^{w_{y \rightarrow y', i}}. \quad (\text{A.8})$$

Let $\mathbf{d} = (\delta_{y \rightarrow y'})_{y \rightarrow y' \in \mathcal{R}}$, $\mathbf{v} = (v_{y \rightarrow y'})_{y \rightarrow y' \in \mathcal{R}}$, and define $\kappa = (k_{y \rightarrow y'})_{y \rightarrow y' \in \mathcal{R}}$ by $k_{y \rightarrow y'} = l_{y \rightarrow y'} / M_{y \rightarrow y'}^J$ where J is the cardinality of $I_{y \rightarrow y'}^+ \cup I_{y \rightarrow y'}^-$. Then $\Sigma(\mathbf{v}) = \Sigma(\mathbf{w})$ and $K = (\kappa, \mathbf{d}, \mathbf{v}) \in \mathcal{K}_H(\mathcal{N})$. Further,

$$l_{y \rightarrow y'} a^{w_{y \rightarrow y'}} = k_{y \rightarrow y'} \prod_{i=1}^n \frac{a_i^{v_{y \rightarrow y', i}}}{\delta_{y \rightarrow y', i} + a_i^{v_{y \rightarrow y', i}}}$$

and similarly for b . It follows that $f_K(a) = f_{\kappa, \mathbf{w}}(a)$ and $f_K(b) = f_{\kappa, \mathbf{w}}(b)$. This proves (ii). To prove (i) we follow the reverse procedure by choosing $M_{y \rightarrow y'}$ and $w_{y \rightarrow y', i}$ to fulfill equation (A.8). \square

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